

Neuroinflammation-Associated Cardiac Alterations: Roles of the Kynurenine Pathway, Autophagy, and Apoptotic Signaling

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Abstract

Neuroinflammation is increasingly recognized as a key driver of peripheral organ dysfunction; however, the molecular mechanisms linking central neuroinflammation to cardiac alterations remain poorly understood. This study investigated whether experimental neuroinflammation induces changes in cardiac kynurenine pathway (KP) metabolism and whether these alterations are associated with inflammation, oxidative stress, autophagy, apoptosis, and cell proliferation. Male Wistar rats were randomly assigned to four groups: SHAM, dexamethasone (DEX), lipopolysaccharide (LPS), and LPS + DEX (n = 6/group). Neuroinflammation was induced by bilateral intracerebroventricular administration of LPS (10 µg total), followed by intraperitoneal DEX (1 mg/kg/day) for seven days. Inflammatory cytokines, oxidative stress markers, cardiac injury biomarkers, KP metabolites, autophagy- and apoptosis-related proteins, and Bax/Bcl-2 gene expression were analyzed in cardiac tissue.

LPS administration significantly increased cardiac TNF-α, IL-1β, NF-κB p65, total oxidant status, oxidative stress index, and quinolinic acid (QA) levels while reducing glutathione, autophagy markers (LC3, ATG5, and Beclin-1), Ki-67, cleaved caspase-3, kynurenine, and the kynurenine/tryptophan ratio. Cardiac Bax and Bcl-2 mRNA expression were both increased in the LPS group, whereas DEX markedly reduced Bax expression and the Bax/Bcl-2 ratio. DEX also attenuated central and peripheral inflammatory responses, reduced oxidative stress and QA accumulation, but failed to restore autophagy- or proliferation-related markers. Interestingly, despite further suppressing apoptotic signaling, the LPS + DEX group exhibited increased circulating cTnI and CK-MB levels. Correlation analyses demonstrated significant associations between KP metabolites and markers of apoptosis, autophagy, and cell proliferation, supporting a close interaction between KP metabolism and cardiac cellular homeostasis.

In conclusion, experimental neuroinflammation induces coordinated inflammatory, oxidative, metabolic, and cellular stress responses in the heart. These findings provide novel evidence for a neuroinflammation–heart axis and suggest that dysregulation of KP metabolism, particularly QA accumulation, together with impaired autophagy, may contribute to cardiac alterations during neuroinflammation. Although DEX attenuated inflammation and pro-apoptotic signaling, its inability to restore autophagy and the paradoxical increase in circulating cardiac injury biomarkers indicate that additional mechanisms may underlie neuroinflammation-associated cardiac injury.

Highlights

- Experimental neuroinflammation induced coordinated inflammatory and oxidative stress responses in cardiac tissue.
- Neuroinflammation disrupted cardiac KP metabolism and promoted quinolinic acid accumulation.
- Impaired autophagy was closely associated with alterations in cardiac kynurenine pathway metabolites.

- DEX reduced inflammation and quinolinic acid accumulation but failed to restore autophagy.
- The findings provide novel evidence supporting a neuroinflammation–heart axis involving KP dysregulation.

1. INTRODUCTION

Neurodegenerative diseases are a group of age-related disorders, including Alzheimer's disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis. Each disease has distinct clinical symptoms and underlying pathological mechanisms that set it apart from the others. Although the clinical symptoms and pathologies of these disorders differ, "neuroinflammation" is a common pathological pathway that triggers and exacerbates their pathologies [1, 2]. While traditional research has treated central and peripheral immune responses as distinct processes, accumulating evidence—particularly in pathological conditions—indicates a bidirectional, dynamic interaction between the central and peripheral immune systems.[3]. The blood–brain barrier (BBB) is a critical structure that protects the central nervous system from potentially neurotoxic and inflammatory components in the blood. However, it has long been recognized that inflammation disrupts BBB integrity [4]. During inflammation, changes in endothelial tight junction organization, increased transcellular transport, leukocyte-endothelial interactions, and dysregulated neurovascular signaling can disrupt BBB integrity. This disruption can alter the transmission of inflammatory signals between central and peripheral compartments [5]. Conversely, centrally initiated inflammatory responses can affect peripheral organs via autonomic nervous system activation, hypothalamic-pituitary-adrenal axis signaling, and systemic release of inflammatory mediators [6].

A key peripheral target of this bidirectional communication is the cardiovascular system [6]. Experimental studies have shown that central inflammatory stimulation can trigger peripheral immune and cardiovascular responses. Intracerebroventricular (ICV) lipopolysaccharide (LPS) administration has been shown to increase circulating interleukin-6 (IL-6) levels and IL-6 expression in peripheral tissues, including the myocardium [7]. It has also been found that central LPS administration activates hypothalamic oxidative and inflammatory signaling, increases sympathetic nervous activity, and elevates heart rate and arterial blood pressure [8]. However, many gaps remain in our understanding of the molecular mechanisms linking neuroinflammation to its cardiac effects.

Recent studies have demonstrated that the kynurenine pathway (KP) plays an important role in the pathophysiology of various neurodegenerative diseases. In particular, neuroinflammation-associated dysregulation of KP metabolites may contribute to neuronal dysfunction and neurotoxicity [9]. Inflammatory and oxidative stimuli promote activation of the kynurenine pathway (KP) primarily through induction of indoleamine 2,3-dioxygenase-1 (IDO1), resulting in enhanced conversion of L-tryptophan (L-Trp) to kynurenine (Kyn). Kyn then serves as a central branching metabolite that can be metabolized through several enzymatic routes: conversion to kynurenic acid (KynA) by kynurenine aminotransferases (KATs), conversion to anthranilic acid by kynureninase followed by downstream formation of quinolinic

acid (QA), or conversion to 3-hydroxykynurenine (3-HK) via kynurenine 3-monooxygenase (KMO). These downstream metabolites exhibit distinct biological activities; QA is generally regarded as a neurotoxic and pro-apoptotic metabolite, whereas KynA is widely recognized for its neuroprotective and anti-inflammatory properties [10]. Although apoptosis is commonly regarded as a marker of cell death, it is also a fundamental physiological mechanism required for normal tissue development and homeostasis. Together with autophagy, which maintains cellular quality control, and proliferation, which supports tissue renewal, apoptosis contributes to preserving cellular and organ integrity. Dysregulation of any of these tightly coordinated processes may disrupt tissue homeostasis and promote inflammation-related tissue injury [11]. Moreover, alterations in the KP have been shown to disrupt autophagy, thereby affecting key cellular processes [12]. Collectively, these findings suggest that central inflammation may exert peripheral effects that lead to alterations in the cardiac KP. However, it remains unclear how neuroinflammation influences cardiac KP metabolism and whether alterations in this pathway affect fundamental cellular processes, including autophagy, apoptosis, and cell proliferation, in the heart.

Dexamethasone (DEX: glucocorticoid receptor (GR) agonist) is widely used as an immunosuppressive and anti-inflammatory agent [13]. Furthermore, in organotypic hippocampal slice cultures (OHSCs), DEX has been shown to modulate IDO expression, suggesting that its anti-inflammatory effects may, at least in part, involve regulation of the KP [14]. Therefore, this study aimed to investigate the role of the KP in neuroinflammation-associated cardiac alterations and to determine how KP dysregulation influences key cellular processes, including autophagy, apoptosis, and cell proliferation, in the heart. In addition, we evaluated whether DEX could modulate these alterations by regulating downstream KP metabolites. We hypothesized that neuroinflammation-induced activation of the KP contributes to cardiac injury by disrupting the balance among autophagy, apoptosis, and cell proliferation, and that DEX exerts cardioprotective effects by restoring KP homeostasis and preserving these cellular processes.

2. MATERIALS and METHODS

2.1. Animal Model and Experimental Procedures

The experimental protocol was reviewed and approved by the Local Animal Experiments Ethics Committee of Akdeniz University (approval no. ID-46/2025.09.B.001). A total of 24 male albino Wistar rats (3 months old, 250–300 g) were supplied by the Akdeniz University Laboratory Animal Center (Antalya, Türkiye). Before initiation of the study, all animals were allowed to acclimate to the housing conditions for 2 days. Throughout the experiment, rats were maintained in groups of four per cage under standardized environmental conditions (23 ± 1 °C, $50 \pm 5\%$ relative humidity) with a 12-h light/12-h dark cycle (lights on from 07:00 to 19:00). Standard laboratory chow and tap water were available ad libitum during the entire experimental period.

Experimental design

The neuroinflammation model was induced by bilateral intracerebroventricular (ICV) administration of lipopolysaccharide (LPS), based on previously reported ICV-LPS protocols with modifications [15, 16].

LPS derived from *Escherichia coli* O111 (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in sterile physiological saline at a concentration of 1 µg/µL. A volume of 5 µL containing 5 µg of LPS was administered into each lateral ventricle. Thus, each rat received 5 µg of LPS per ventricle, corresponding to a total bilateral dose of 10 µg.

Dexamethasone (DEX; purity ≥98%) was obtained from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China) and prepared in physiological saline. DEX was administered intraperitoneally (i.p.) at a dose of 1 mg/kg once daily for 7 consecutive days, beginning 24 h after the ICV procedure [17].

The animals were randomly allocated to four experimental groups, with six rats in each group:

1. Sham group (SHAM): Rats received bilateral ICV injections of sterile saline at a volume of 5 µL per ventricle, followed by an equivalent volume of saline administered i.p. once daily for 7 consecutive days.
2. Dexamethasone group (DEX): Rats received bilateral ICV injections of sterile saline at a volume of 5 µL per ventricle, followed by DEX at a dose of 1 mg/kg i.p. once daily for 7 consecutive days.
3. Lipopolysaccharide group (LPS): Rats received bilateral ICV injections of LPS at a dose of 5 µg in 5 µL of sterile saline per ventricle, followed by an equivalent volume of saline administered i.p. once daily for 7 consecutive days.
4. LPS plus dexamethasone group (LPS+DEX): Rats received bilateral ICV injections of LPS at a dose of 5 µg in 5 µL of sterile saline per ventricle, followed by DEX at a dose of 1 mg/kg i.p. once daily for 7 consecutive days.

Intracerebroventricular administration

The rats were anesthetized with ketamine/xylazine at 80 and 5 mg/kg, respectively, administered i.p., and then secured in a stereotaxic apparatus. After a midline sagittal scalp incision, bilateral burr holes were drilled above the lateral ventricles. The stereotaxic coordinates were anteroposterior -0.8 mm, mediolateral ±1.5 mm, and dorsoventral -3.6 mm relative to bregma. Using a 10-µL Hamilton microsyringe, 5 µL of an LPS solution containing 5 µg of LPS was infused into each lateral ventricle at 1 µL/min for 5 min. Rats in the SHAM and DEX groups received equivalent volumes of sterile physiological saline using the same coordinates, infusion rate, and procedure. After ICV administration, the scalp incision was closed with sutures, and topical antibiotic ointment was applied to the incision site [15]. After the experimental procedure was completed, the animals were sacrificed, and tissue and blood samples were taken.

The seven-day timeframe following LPS administration was chosen to assess persistent neuroinflammatory and neurobiological changes rather than immediate acute phase-specific responses. Jung et al. demonstrated that LPS-induced hippocampal microglial activation was followed by GABAergic synaptic abnormalities detectable 4–6 days after LPS exposure, and that memory impairment persisted for at least 6 days [18]. Similarly, behavioral, neurochemical, and histopathological

changes were assessed 7 days after ICV LPS administration in a rat model of neuroinflammation. Memory impairment was detected even after recovery from overt systemic signs of inflammation 7 days after systemic LPS administration [19]. Therefore, the assessment on day 7 aimed to reduce the likelihood that the findings would predominantly reflect immediate disease-related or acute perioperative responses, while ensuring that the persistent inflammatory, synaptic, and cognitive consequences of LPS exposure remained detectable [18-20].

DEX treatment was initiated 24 hours after ICV administration to establish a post-traumatic therapeutic intervention rather than a prophylactic treatment paradigm. To provide anti-inflammatory intervention throughout the range in which LPS-induced neuroinflammatory and synaptic changes develop and persist, treatment was continued once daily until the 7-day endpoint. The 1 mg/kg i.p. dose was chosen based on previous experimental evidence demonstrating the biological and anti-inflammatory activity of this dose in rat models of CNS injury. It has also been noted that DEX has been shown in rat intracerebral injury models to reduce cerebral edema, inflammatory cell accumulation, and neurological impairment [21, 22]. Furthermore, experimental evidence suggests that LPS and DEX can produce different tissue- and glial cell-specific changes in kynurenine pathway metabolism, providing a mechanistic rationale for investigating the effects of DEX on central and peripheral KP responses in this study [23]. Therefore, the chosen DEX regimen aimed to provide continuous glucocorticoid exposure throughout the 7-day experimental period and to evaluate both its anti-inflammatory effects and its potential site-specific modulation of KP.

2.2 Biochemical analysis

Excised brain and heart tissue samples were first rinsed with ice-cold saline, weighed, and homogenized on ice in phosphate-buffered saline (PBS) at a 1:9 (w/v) ratio using a glass homogenizer. The homogenates were centrifuged at $10,000 \times g$ for 5 minutes at 4°C , and the supernatants were collected for biochemical analyses. Plasma samples were obtained by centrifuging whole blood at $1,500 \times g$ for 15 minutes. All samples were stored at -80°C for subsequent biochemical analysis [24].

Assessment of Pro-inflammatory Markers: Cardiac levels of tumor necrosis factor-alpha (TNF- α), interleukin-1 beta (IL-1 β), nuclear factor kappa B (NF- κ B), and the nuclear factor kappa B p65 (RelA) subunit (p65) in cardiac tissue, and IL-1 β levels in brain tissues, were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits.

Assessment of Oxidative Stress Related Parameters: Cardiac tissue levels of total oxidant status (TOS) and total antioxidant status (TAS) were determined using commercially available colorimetric assay kits (Rel Assay Diagnostics, Gaziantep, Türkiye) according to the manufacturer's instructions. TOS was quantified by measuring the oxidation of the ferrous ion–o-dianisidine complex to ferric ions by oxidant species present in the samples. Under acidic conditions, the resulting ferric ions react with xylenol orange to generate a colored complex, the absorbance of which was recorded at 530 nm. The measured signal is proportional to the overall oxidant capacity of the sample. TAS was assessed using the ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolorization assay. Antioxidants

contained in the samples reduce the ABTS radical, resulting in a decline in absorbance measured at 660 nm, which reflects the total antioxidant capacity. The oxidative stress index (OSI) was calculated as the ratio of TOS to TAS and expressed as arbitrary units using the following equation: $OSI = TOS (\mu\text{mol H}_2\text{O}_2 \text{ equivalent/L}) / TAS (\mu\text{mol Trolox equivalent/L})$ [25]. Cardiac Glutathione (GSH) levels were measured using commercial ELISA kits.

Measurement of Plasma Cardiac Injury Biomarkers: Circulating levels of creatine kinase (CK) CK and creatine kinase–MB (CK-MB) were measured by colorimetric methods using a Mindray BS400 automated clinical biochemistry analyzer. Cardiac troponin I (cTnI) and lactate dehydrogenase (LDH) cTnI and LDH concentrations in plasma were determined with commercially available ELISA kits following the manufacturers' instructions.

The relative index of CK mass is determined by the use of the formula:

Relative index = $CK-MB/TotalCK \times 100$ [26]

Assessment of KP Pathway Metabolites: Trp, Kyn, KYNA, and QA levels in cardiac tissue samples were measured using commercial ELISA kits. The change in the Kyn/Trp ratio reflects the degradation rate of Trp, and therefore, the Kyn/Trp ratio is used to determine IDO activity [27-29].

Assessment of Autophagy and Apoptosis-Related Parameters and a Cell Proliferation

Marker: Autophagy was evaluated by measuring beclin 1, autophagy-related protein 5 (ATG5), and Microtubule-associated protein 1 light chain 3 (LC3) [30]; apoptosis was assessed by cleaved caspase-3 [28, 29]; and cell proliferation was determined by Ki-67 [31] levels using commercial ELISA kits.

The catalog numbers of the assay kits used were as follows: TNF- α ELISA Kit Catalog no: E0764Ra, IL-1 β ELISA Kit Catalog no: E0119Ra, NF- κ B ELISA Kit Catalog no: E0287Ra, NF- κ B-p65 ELISA Kit Catalog no: E0290Ra, Troponin I ELISA Kit Catalog no: E0305Ra, LDH ELISA Kit Catalog no: E0530Ra, LC3B ELISA Kit Catalog no: E1546Ra, ATG5 ELISA Kit Catalog no: E1547Ra, Beclin 1 ELISA Kit Catalog no: E0897Ra, Ki-67 ELISA Kit Catalog no: E0063Ra, Cleaved-caspase 3 ELISA Kit Catalog no: E2444Ra, GSH ELISA Kit, Catalog no: EA0113Ra; Trp ELISA Kit Catalog no: E2630, Kynurenine ELISA Kit Catalog no: E1203Ra, Kynurenic acid ELISA Kit Catalog no: E2527Ra Quinolinic acid ELISA Kit Catalog no: EA005Ge. All kits were obtained from Bioassay Technology Laboratory (Shanghai, China).

The results obtained from cardiac tissue homogenates were normalized to total protein concentration to minimize variability among samples [28, 29, 32]. Protein levels were determined by a modified Bradford assay using Coomassie Plus Protein Assay Reagent (Pierce Chemical Company, Rockford, IL, USA) with bovine serum albumin as the reference standard. Optical density was recorded at 595 nm, and protein concentrations were calculated from the corresponding standard curve [33].

2.3. Measurement of *Bax* and *Bcl-2* mRNA Expression by RT-qPCR

RNA Isolation and cDNA Synthesis

Approximately 30 mg of cardiac tissue from each animal was processed for total RNA isolation using the ExiPrep™ Tissue Total RNA Kit (K-3325, Bioneer Corporation, Daejeon, Republic of Korea). Cardiac samples were first disrupted in Tissue Lysis Buffer with a BIOPREP-24 homogenization system (Allsheng, Hangzhou, China). After centrifugation, the clarified lysate was loaded into ExiPrep™ RNA extraction cartridges, and RNA purification was completed using the automated extraction program recommended by the manufacturer. RNA yield and purity were assessed with a DeNovix microvolume spectrophotometer (DeNovix Inc., Wilmington, DE, USA). Samples were considered suitable for downstream analysis only when their A260/A280 absorbance ratios ranged from 1.8 to 2.0 [34]. Before reverse transcription, all RNA preparations were adjusted to 100 ng/μL. For each sample, 10 μL of normalized RNA, corresponding to 1 μg of total RNA, was combined with 3 μL of a gene-specific reverse primer solution at a concentration of 10 pmol. This mixture was heated at 70°C for 5 min and rapidly cooled on ice. Nuclease-free water was then added to obtain a final volume of 50 μL, and the mixture was transferred into AccuPower® RT PreMix tubes (K-2041, Bioneer Corporation, Daejeon, Republic of Korea). Complementary DNA synthesis was carried out at 42°C for 60 min, followed by incubation at 94°C for 5 min to terminate reverse transcriptase activity. The resulting cDNA samples were maintained at –20°C until quantitative real-time PCR analysis.

Quantitative Real-Time PCR

Gene expression analysis was performed using SYBR Green-based quantitative real-time PCR. Each reaction was prepared in a total volume of 20 μL and consisted of 10 μL of SYBR Green qPCR Master Mix (Hibrigen, Istanbul, Türkiye), 5 μL of cDNA, 2 μL of combined forward and reverse primers at 10 pmol each, and 3 μL of nuclease-free water. Amplification was conducted with a Q2000B Real-Time PCR System (LongGene Scientific Instruments Co., Ltd., Hangzhou, China). The cycling program began with an initial denaturation step at 95°C for 3 min, followed by 35 amplification cycles consisting of denaturation at 95°C for 30 s and combined annealing and extension at 54°C for 25 s. The oligonucleotide sequences used for amplification are provided in Table 1. Expression data were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH). For each sample, ΔCt was calculated as the difference between the cycle threshold of the target gene and that of GAPDH. Relative transcript abundance was subsequently estimated using the 2^{–ΔΔCt} approach, with the control group selected as the reference calibrator [34].

Table 1. The primer sequences used for RT-qPCR

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
<i>Bcl-2</i>	GTGGATGACTGAGTACCT	CCAGGAGAAATCAAACAGAG
<i>Bax</i>	ATGGAGCTGCAGAGGATGA	CCAGTTTGCTAGCAAAGTAG
<i>GAPDH</i>	GGCAAGTTCAACGGCACAG	CGCCAGTAGACTCCACGACAT

Abbreviations: *Bcl-2*, B-cell lymphoma 2; *Bax*, BCL2-associated x; **GAPDH**, glyceraldehyde 3-phosphate dehydrogenase; **RT-qPCR**, Reverse transcription quantitative real-time polymerase chain reaction

2.4. Statistical analysis

The sample size (n = 6 per group) was determined by an a priori power analysis to provide sufficient statistical power for detecting biologically relevant differences among the experimental groups. Statistical analyses were conducted using IBM SPSS Statistics for Windows (version 18.0; SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± standard deviation (SD). The normality of continuous variables was evaluated using the Shapiro–Wilk test, while homogeneity of variance was assessed with Levene’s test. Variables fulfilling the assumptions of parametric analysis were analyzed by one-way analysis of variance (ANOVA), followed by Tukey’s post hoc test for multiple group comparisons. Because *Bcl-2* mRNA expression data did not satisfy the assumptions required for parametric testing, the prespecified pairwise comparisons (SHAM vs. LPS and LPS vs. LPS+DEX) were performed using the Mann–Whitney U test. Relationships among KP metabolites, inflammatory and oxidative stress biomarkers, and markers related to apoptosis, autophagy, and cell proliferation were examined using Spearman’s rank correlation coefficient. Statistical significance was defined as a two-sided p value of < 0.05.

3. RESULTS

3.1. Changes of Pro-inflammatory Parameters

Results for pro-inflammatory parameters in the experimental groups are presented in Table 2. Hippocampus IL-1β levels in the LPS group were higher than in the Sham group (p < 0.001), and DEX administration was reduced in the LPS+DEX group compared with the LPS group (p<0.01). Temporal cortex IL-1β levels in the LPS group were higher than in the Sham group (p < 0.001), and DEX administration was reduced in the LPS+DEX group compared with the LPS group (p<0.01). Frontal cortex IL-1β levels in the LPS group were higher than in the Sham group (p < 0.001), and DEX administration was reduced in the LPS+DEX group compared with the LPS group (p<0.01).

Cardiac IL-1β levels were higher in the LPS group than in the Sham group (p < 0.01). Cardiac IL-1β levels were lower in the LPS+DEX group than in the LPS group (p < 0.01). Cardiac TNF-α levels in the LPS group were also higher than in the Sham group (p < 0.01), and DEX administration reduced levels in the LPS+DEX group compared with the LPS group (p < 0.01). No significant difference was observed in plasma Cardiac NF-κB levels among the groups. Cardiac NF-κB p65 levels in the LPS group were higher than in the Sham group (p < 0.01), and DEX administration reduced levels in the LPS+DEX group compared with the LPS group (p < 0.05).

Table 2:Evaluation of Changes in Pro-inflammatory Parameters Between Groups

	SHAM	DEX	LPS	LPS+DEX
Hippocampus IL-1β Levels (ng/mg protein)	0.454 $\pm$ 0.222	0.504 $\pm$ 0.321	1.316 $\pm$ 0.281***	0.612 $\pm$ 0.213 $\square\square$
Temporal Cortex IL-1β Levels (ng/mg protein)	0.309 $\pm$ 0.176	0.294 $\pm$ 0.228	1.114 $\pm$ 0.250***	0.381 $\pm$ 0.162 $\square\square$
Frontal Cortex IL-1β Levels (ng/mg protein)	0.464 $\pm$ 0.227	0.519 $\pm$ 0.321	1.314 $\pm$ 0.289***	0.627 $\pm$ 0.236 $\square\square$
Cardiac IL-1β Levels (ng/mg protein)	0.654 $\pm$ 0.222	0.779 $\pm$ 0.330	1.266 $\pm$ 0.281**	0.642 $\pm$ 0.213 $\square\square$
Cardiac TNF-α Levels (ng/g protein)	18.639 $\pm$ 6.213	29.390 $\pm$ 4.093	31.933 $\pm$ 9.213**	23.132 $\pm$ 8.456 $\square\square$
Cardiac NF-κB Levels (ng/g protein)	0.112 $\pm$ 0.058	0.080 $\pm$ 0.026	0.071 $\pm$ 0.033	0.034 $\pm$ 0.014
Cardiac NF-κB p65 Levels (ng/g protein)	0.174 $\pm$ 0.028	0.212 $\pm$ 0.092	0.285 $\pm$ 0.053**	0.182 $\pm$ 0.068 $\square$

Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by the Tukey post hoc test for all parameters except cardiac NF- κ B p65 levels. Statistical analysis was performed using Kruskal–Wallis test was followed by Mann–Whitney U tests for cardiac NF- κ B p65 levels. All values are mean $\pm$ SD. n = 6 for each group. *, p<0.05 vs SHAM; **, p<0.01 vs SHAM; ***, p<0.001 vs SHAM; $\square$, p<0.05 vs LPS; $\square\square$, p<0.01 vs LPS; $\square\square\square$, p<0.001 vs LPS

Abbreviations: **TNF- α** , Tumor necrosis factor-alpha; **IL-1 β** , Interleukin-1 beta; **NF- κ B**, Nuclear factor kappa B; **P65**, NF- κ B p65 subunit.

3.2. Changes of Oxidative Stress Parameters

Results of oxidative stress-related parameters for the experimental groups are presented in Table 3. No significant difference was observed in cardiac TAS levels among the groups. Cardiac TOS levels were higher in the LPS group than in the Sham group (p<0.01), and DEX administration reduced TOS levels in the LPS+DEX group compared with the LPS group (p<0.01). Cardiac OSI levels were higher in the LPS group than in the Sham group (p<0.05), and DEX administration reduced OSI levels in the LPS+DEX group compared with the LPS group (p<0.05). Cardiac GSH levels were lower in the LPS group than in the Sham group (p<0.05), and DEX administration further reduced GSH levels in the LPS+DEX group compared with the LPS group; however, this decrease did not reach statistical significance. Plasma TAS levels were higher in the LPS group than in the Sham group (p<0.05).

Plasma TOS levels were higher in the LPS group than in the Sham group ($p<0.05$), and DEX administration was lower in the LPS+DEX group than in the LPS group ($p<0.05$). No significant difference was observed in plasma OSI levels among the groups.

Table 3:Evaluation of Changes in Oxidative Stres Related Parameters Between Groups

	SHAM	DEX	LPS	LPS+DEX
Cardiac TAS Levels (mmol Trolox/ g protein)	11.165±1.793	12.107±1.484	11.575±1.804	10.909±0.556
Cardiac TOS Levels ($\mu\text{mol H}_2\text{O}_2$ Eq/ g protein)	0.311±0.034	0.244±0.114	0.479±0.020**	0.311±0.102 ^{§§}
Cardiac OSI Levels (Arbitrary units)	0.030±0.005	0.019±0.008	0.043±0.0045*	0.030±0.010 [§]
Cardiac GSH Levels (ng/g protein)	237.83±66.22	294.31±88.44	179.15±13.46*	158.63±33.69
Plasma TAS Levels (mmol Trolox/ L)	1.900±0.130	2.071±0.241	2.588±0.651*	2.168±0.75
Plasma TOS Levels ($\mu\text{mol H}_2\text{O}_2$ Eq/ g protein)	3.394±1.256	4.115±0.455	5.150±0.610*	3.648±0.973 [§]
Plasma OSI Levels (Arbitrary units)	2.456±0.739	2.202±0.591	2.552±0.284	1.877±0.860

Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. All values are mean $\pm$ SD. n = 6 for each group. *, $p<0.05$ vs SHAM; **, $p<0.01$ vs SHAM; [§], $p<0.05$ vs LPS; ^{§§}, $p<0.01$ vs LPS. **Abbreviations:** **TAS**, Total antioxidant status; **TOS**, Total oxidant status; **OSI**, Oxidative stress index; **GSH**, Glutathione.

3.2. Changes of Plasma Cardiac Injury Biomarkers

Plasma cardiac injury parameter results for the experimental groups are presented in Table 4. No significant differences were observed in plasma CK and LDH levels among the groups ($p>0.05$). No significant differences were observed in plasma CK-MB levels between the LPS and Sham groups. DEX administration increased CK-MB levels in the LPS+DEX group compared with the LPS group ($p<0.001$). No significant differences were observed in relative CK-MB index levels between the LPS and Sham groups. DEX administration increased the CK-MB relative index in the LPS+DEX group compared with the LPS group ($p<0.05$). No significant differences were observed in plasma cTnI levels between the LPS and Sham groups. DEX administration increased cTnI levels in the LPS+DEX group compared with the LPS group ($p<0.05$).

Table 4: Plasma Cardiac Injury Biomarkers

	SHAM	DEX	LPS	LPS+DEX
Plasma CK Levels (U/L)	895.192±392.843	1162.34±134.82	890.35±61.180	1083±231.908
Plasma CK-MB Levels (U/L)	696.425±138.96	729.570±57.084	571.326±71.957	890.206±102.483 ^{***}
CK-MB Relative Index (%)	88.646±32.141	63.185±5.998	85.017±19.060	75.221±21.432*
Plasma cTnI Levels (mg/ml)	300.239±53.157	321.257±84.772	293.945±66.992	369.581±31.978*
Plasma LDH Levels (U/L)	125.517±38.346	109.428±36.260	149.667±61.549	183.297±30.271

Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by the Tukey post hoc test for all parameters except cTnI levels and CK-MB relative index. Statistical analysis was performed using Kruskal–Wallis test was followed by Mann–Whitney U tests for cTnI levels and CK-MB relative index. All values are mean ± SD. n = 6 per group.

*, p<0.05 vs SHAM; ^{***}, p<0.001 vs LPS. **Abbreviations:** **CK**, Creatine kinase; **CK-MB**, Creatine kinase–myocardial band; **cTnI**, Cardiac troponin I; **LDH**, Lactate dehydrogenase

3.3. Changes of KP Metabolites

Cardiac Trp levels were higher in the LPS group ($11.503 \pm 2.335 \mu\text{mol/g protein}$) and the LPS+DEX group ($11.998 \pm 2.423 \mu\text{mol/g protein}$) than in the Sham group ($5.765 \pm 0.974 \mu\text{mol/g protein}$) (p<0.001 for both comparisons). There was no difference between the Sham and DEX groups ($7.013 \pm 1.433 \mu\text{mol/g protein}$) (Figure 2A)

Cardiac KYN levels were lower in the LPS group ($0.304 \pm 0.074 \mu\text{g/g protein}$) than in the Sham group ($0.485 \pm 0.097 \mu\text{g/g protein}$) (p<0.01). However, there was no difference between the Sham and DEX groups ($0.416 \pm 0.064 \mu\text{g/g protein}$) or between the LPS and LPS+DEX groups ($0.419 \pm 0.109 \mu\text{g/g protein}$) (Figure 2B).

Cardiac KYN/Trp levels were lower in the LPS group ($0.0289 \pm 0.008 \mu\text{g}/\mu\text{mol}$) than in the Sham group ($0.0644 \pm 0.037 \mu\text{g}/\mu\text{mol}$) (p<0.05). However, there was no difference between the Sham and DEX groups ($0.0605 \pm 0.012 \mu\text{g}/\mu\text{mol}$) or between the LPS and LPS+DEX groups ($0.0365 \pm 0.014 \mu\text{g}/\mu\text{mol}$) (Figure 2C).

Cardiac KYNA levels were higher in the DEX group ($3.696 \pm 0.592 \mu\text{g/g protein}$) than in the Sham group ($2.858 \pm 0.411 \mu\text{g/g protein}$) (p<0.05). However, there was no difference between the Sham and LPS groups ($2.870 \pm 0.498 \mu\text{g/g protein}$) or between the LPS and LPS+DEX groups ($2.641 \pm 0.363 \mu\text{g/g protein}$) (Figure 2D).

Cardiac QA levels were higher in the LPS group ($0.234 \pm 0.032 \mu\text{g/g protein}$) than in the Sham ($0.143 \pm 0.012 \mu\text{g/g protein}$) and LPS+DEX group ($0.1694 \pm 0.015 \mu\text{g/g protein}$) ($p < 0.001$ for both comparisons). However, there was no difference between the Sham and DEX groups ($0.144 \pm 0.015 \mu\text{g/g protein}$) (Figure 2E).

3.4.Changes of Autophagy, Apoptosis, and Cell Proliferation Related Parameters

Changes in autophagy, apoptosis, and cell proliferation-related parameters are presented in Table 5. Cardiac LC3B, ATG5, and Beclin-1 levels in the LPS group were lower than those in the Sham group ($p < 0.01$, $p < 0.05$, and $p < 0.001$, respectively). However, DEX administration did not significantly alter LC3B, ATG5, and Beclin-1 in the LPS+DEX group compared with the LPS group. Cardiac Ki-67 levels in the LPS group were lower than those in the Sham group ($p < 0.001$). Cardiac cleaved caspase-3 levels in the LPS groups were lower than in the Sham group ($p < 0.01$). DEX administration also decreased in the LPS+DEX group compared with the LPS group ($p < 0.05$).

Table 5:Evaluation of Changes in Autophagy, Apoptosis and Proliferation Parameters Between Groups

	SHAM	DEX	LPS	LPS+DEX
Cardiac LC3 Levels (ng/g protein)	11.054 $\pm$ 3.193	12.204 $\pm$ 4.048	5.064 $\pm$ 1.695**	4.809 $\pm$ 1.622
Cardiac ATG-5 Levels (ng/g protein)	13.959 $\pm$ 7.288	15.713 $\pm$ 10.057*	5.394 $\pm$ 1.688***	5.876 $\pm$ 4.368
Cardiac Beclin 1 Levels (ng/mg protein)	0.179 $\pm$ 0.0517	0.104 $\pm$ 0.050	0.055 $\pm$ 0.028*	0.036 $\pm$ 0.019
Cardiac Ki-67 Levels (ng/mg protein)	0.367 $\pm$ 0.037	0.356 $\pm$ 0.045	0.209 $\pm$ 0.082***	0.183 $\pm$ 0.040
Cardiac cleaved caspase-3 Levels (ng/g protein)	55.662 $\pm$ 9.533	50.515 $\pm$ 8.748	37.835 $\pm$ 6.249**	25.756 $\pm$ 5.622 [§]

Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by the Tukey post hoc test except cardiac cleaved caspase3- levels. Statistical analysis was performed using Kruskal–Wallis test was followed by Mann–Whitney U tests for cleaved caspase3- levels. All values are presented as mean $\pm$ SD. n = 6 per group. *, $p < 0.05$ vs SHAM; **, $p < 0.01$ vs SHAM; ***, $p < 0.001$ vs SHAM; [§], $p < 0.05$ vs LPS. **Abbreviations:** **LC3**, Microtubule-associated protein 1 light chain 3; **ATG-5**, autophagy-related proteins-5;**Ki-67**, Marker of proliferation Ki-67 gene.

3.5. Bax and Bcl-2 mRNA Expression in the Experimental Groups

Bax gene expression differed significantly among the experimental groups. *BAX* mRNA expression was significantly higher in the LPS group (2.00 ± 1.08) than in the SHAM group (0.60 ± 0.31) ($p < 0.001$). DEX treatment alone (1.50 ± 0.36) did not significantly alter *Bax* expression compared with the Sham group ($p > 0.05$). In contrast, *Bax* expression was markedly reduced in the LPS+DEX group (0.20 ± 0.11) compared with the LPS group ($p < 0.0001$), representing the lowest level among all groups (Figure 3A).

Bcl-2 gene expression was significantly increased in the LPS group (3.32 ± 0.92) compared with the Sham group (1.29 ± 0.34) ($p < 0.01$). Although *Bcl-2* expression was higher in the LPS+DEX group (5.29 ± 2.09) than in the LPS group (3.32 ± 0.92), this difference did not reach statistical significance ($p > 0.05$). *Bcl-2* expression in the DEX group (2.81 ± 1.66) was not significantly different from that in the SHAM group ($p > 0.05$) (Figure 3B).

The *Bax/Bcl-2* ratio was significantly reduced in the LPS+DEX group (0.040 ± 0.021) compared with the LPS group (0.579 ± 0.185 , $p < 0.01$), whereas no significant differences were detected among the SHAM (0.436 ± 0.123), DEX (0.712 ± 0.363), and LPS groups (Figure 3C).

3.6. Correlations Among Cardiac Kynurenine Pathway Metabolites, Oxidative Stress, Apoptosis, and Autophagy Markers

Spearman correlation analysis revealed several significant associations among cardiac kynurenine pathway metabolites, oxidative stress, apoptosis, proliferation, and autophagy markers (Table 6). *Bax* fold change showed a strong positive correlation with the *Bax/Bcl-2* ratio ($p < 0.001$) and a moderate positive correlation with LC3 expression ($p < 0.05$). In contrast, BCL-2 fold change was negatively correlated with the Ki-67 ($p < 0.05$), cleaved caspase-3 ($p < 0.01$), and Beclin-1 ($p < 0.05$). The *Bax/Bcl-2* ratio exhibited positive correlations with Ki-67 ($p < 0.01$), cleaved caspase-3 ($p < 0.01$), LC3 ($p < 0.05$), Beclin-1 ($p < 0.05$), and ATG5 ($p < 0.05$).

Among KP metabolites, Trp was positively correlated with QA ($p < 0.05$) and negatively correlated with Ki-67 ($p < 0.01$), cleaved caspase-3 ($p < 0.01$), LC3 ($p < 0.01$), Beclin-1 ($p < 0.01$), and ATG5 ($p < 0.05$). QA was positively associated with TOS ($p < 0.05$), and Kyn was positively associated with KYNA ($p < 0.01$) and Ki-67 ($p < 0.05$). KYNA showed positive correlations with Ki-67 ($p < 0.01$), cleaved caspase-3 ($p < 0.05$), LC3 ($p < 0.05$), Beclin-1 ($p < 0.05$), and ATG5 ($p < 0.01$). Ki-67 was strongly positively correlated with cleaved caspase-3 ($p < 0.001$), LC3 ($p < 0.001$), Beclin-1 ($p < 0.001$), and ATG5 ($p < 0.01$). Moreover, cleaved caspase-3 was positively correlated with LC3 ($p < 0.001$), Beclin-1 ($p < 0.001$), and ATG5 ($p < 0.01$), while LC3 was positively associated with Beclin-1 ($p < 0.001$) and ATG5 ($p < 0.01$). Beclin-1 also showed a positive correlation with ATG5 ($p < 0.01$).

Table 6:Correlation Matrix of Cardiac Biochemical and Molecular Markers

4. DISCUSSION

4.1 Neuroinflammation-Induced Cardiac Inflammation and Oxidative Stress

LPS is a potent endotoxin widely used to induce experimental neuroinflammation because it promotes the release of pro-inflammatory cytokines and triggers neurotoxicity [15, 16]. Accordingly, the increased IL-1 β levels observed in the hippocampus, temporal cortex, and frontal cortex of the LPS group compared with the SHAM group confirmed successful establishment of the neuroinflammation model. These brain regions are commonly evaluated in neuroinflammation studies because they represent different but complementary aspects of brain function, enabling a comprehensive assessment of region-specific inflammatory responses [35-38]. Therefore, the observed increase in IL-1 β across these regions indicates that LPS administration by ICV effectively induced neuroinflammation, which may provide a valid experimental model for investigating its downstream effects on peripheral organs, including the heart.

Additionally, our results are consistent with previous studies demonstrating a bidirectional, dynamic interaction between the central and peripheral immune systems [3, 4], as cardiac TNF- α , IL-1 β , and p65 levels in the LPS group were higher than in the SHAM group. In this regard, our findings are in agreement with previous studies showing that central inflammatory activation extends to peripheral organs, including the heart, leading to myocardial inflammation and cellular injury [39]. NF- κ B comprises five structurally related subunits, including p50, p52, p65 (RelA), RelB, and c-Rel. LPS-induced activation promotes dissociation and nuclear translocation of the canonical p50/p65 heterodimer, thereby increasing inflammatory cytokine expression and amplifying the inflammatory response [40]. Assis et al. demonstrated that prolonged NF- κ B activation, characterized by increased p65 expression and elevated nuclear p65 levels, contributes to sustained inflammatory signaling [41]. Therefore, our results suggest that NF- κ B activation may be associated with increased inflammatory cytokine expression in the cardiac tissue of the LPS group. Furthermore, the observation that DEX reduced inflammatory cytokine levels in both the examined brain regions and cardiac tissue in the LPS+DEX group. Consistent with previous studies, these results further support the therapeutic potential of DEX in attenuating both central and peripheral inflammation [42], likely through suppression of NF- κ B-mediated signaling pathways and inhibition of inflammatory cytokine expression in cardiac tissue [43].

There is a bidirectional relationship between the NF- κ B signaling pathway and oxidative stress: ROS production triggers NF- κ B activation, and activated NF- κ B further exacerbates oxidative stress by upregulating pro-inflammatory and oxidant-producing pathways [44]. Similarly, our results showed that the LPS group had higher TOS and OSI levels in cardiac tissue than the SHAM group. Additionally, decreased cardiac GSH levels in the LPS group compared with the Sham group highlight the critical role of GSH in maintaining cardiac redox homeostasis. Consistent with these findings, in an LPS-induced stress model, decreased GSH-Px levels in cardiac tissue were observed, suggesting a weakening of the myocardium's antioxidant defense against oxidative stress and increased susceptibility to oxidative damage [45]. Additionally, our results suggest that the protective effects of DEX on redox balance may be associated with reduced ROS production rather than with increased total antioxidant capacity, because DEX administration decreased cardiac TOS and OSI levels in the LPS+DEX group compared with the LPS group, whereas TAS levels remained unchanged. Supporting this view, a previous study showed that DEX has been shown to suppress ROS production, nitric oxide release, and inflammatory responses

in activated microglial cells [46]. Collectively, our findings suggest that experimental neuroinflammation may extend beyond the central nervous system and trigger inflammatory and oxidative stress responses in cardiac tissue. Despite growing evidence supporting this association, the molecular pathways that mediate the interaction between central neuroinflammation and peripheral inflammatory responses [3, 4] remain poorly understood.

4.2 Kynurenine Pathway Dysregulation in the Neuroinflammation–Heart Axis

Our results showed that the cardiac Kyn/Trp ratio was significantly decreased in the LPS group, suggesting reduced IDO activity, as the Kyn/Trp ratio is widely used as an indirect marker of IDO activity [27-29]. Consistent with this finding, cardiac Trp levels were increased, whereas Kyn levels were decreased in the LPS group. Despite the reduced Kyn/Trp ratio, cardiac QA levels were significantly increased, suggesting that its accumulation may not be solely dependent on IDO activity but may instead reflect alterations in downstream KP enzymes, impaired QA metabolism, or both [47]. To the best of our knowledge, however, this is the first study to demonstrate QA accumulation in cardiac tissue in an experimental model of neuroinflammation. The molecular mechanisms underlying this metabolite accumulation remain unclear and warrant further investigation, particularly regarding downstream KP enzymes and QA metabolism.

The concurrent increase in cardiac IL-1 β , TNF- α , and QA levels observed in the present study, together with the reduction in all three markers following DEX treatment, is noteworthy, as previous studies have demonstrated close functional interactions among these mediators. Pro-inflammatory cytokines, including TNF- α , have been shown to increase QA production under inflammatory conditions [48]. Additionally, IL-1 β has been shown to potentiate QA-induced neurotoxicity, while QA itself can further enhance IL-1 β expression, suggesting a positive inflammatory feedback loop [49, 50]. Furthermore, Jeon et al. demonstrated that DEX suppresses LPS-induced IL-1 β gene expression by inhibiting NF- κ B/Rel and AP-1 activation, thereby reducing the nuclear translocation of NF- κ B subunits, including p65 [43]. Additionally, previous reports have suggested that QA can activate the NF- κ B signaling pathway by promoting nuclear translocation of the p65 subunit [51]. Consistent with these findings, cardiac QA and p65 levels were concurrently increased in the LPS group and decreased following DEX treatment, suggesting a close relationship between QA accumulation and NF- κ B activation during neuroinflammation. Additionally, our previous studies have suggested that alterations in the KP may be associated with cardiac injury [29]. Taken together, the present findings suggest that neuroinflammation-associated alterations in the KP may modulate cardiac cellular processes, including autophagy, apoptosis, and cell proliferation.

4.3 Neuroinflammation-Induced Alterations in Cardiac Cell Survival and Autophagy

Previous studies have demonstrated that QA induces mitochondrial dysfunction, increases the *Bax/Bcl-2* ratio, promotes cytochrome c release, and activates caspase-3, ultimately leading to apoptosis [52, 53]. In the present study, cardiac QA and *Bax* levels were significantly increased in the LPS group; however, unlike previous reports, *Bcl-2* levels were also significantly increased, whereas cleaved caspase-3 levels

were significantly decreased. These findings suggest that although molecular changes associated with pro-apoptotic signaling were evident, execution of the apoptotic program may have been limited by a compensatory increase in *Bcl-2*, thereby preventing caspase-3-dependent apoptosis. Supporting this interpretation, correlation analysis revealed a significant negative correlation between cleaved caspase-3 and *Bcl-2* expression, and a significant positive correlation between cleaved caspase-3 and the *Bax/Bcl-2* ratio.

Autophagy plays a critical role in maintaining cardiac homeostasis by removing damaged proteins and organelles and promoting cell survival under stress conditions [54]. Although autophagy and apoptosis are often considered opposing processes, accumulating evidence indicates that they are closely interconnected and may be coordinately regulated depending on the cellular context [55]. In the present study, LPS administration significantly reduced cardiac expression of LC3, ATG5, and Beclin-1, indicating impaired autophagic activity. This reduction was accompanied by lower Ki-67 levels, suggesting diminished cellular renewal or proliferative capacity. Therefore, the concomitant reductions in autophagy- and apoptosis-related markers observed in the LPS group may reflect impaired cellular turnover rather than activation of a classical cell death program.

Recent evidence indicates that the KP is closely interconnected with cellular quality-control mechanisms, particularly autophagy. Alterations in KP metabolism have been shown to influence autophagic flux through multiple mechanisms, including activation of the aryl hydrocarbon receptor (AhR), whereas QA has been reported to disrupt autophagy-dependent cellular homeostasis under inflammatory conditions [56, 57]. Consistent with these observations, the present study demonstrated that a significant negative correlation between QA and LC3 further supports a potential relationship between altered KP metabolism and impaired cardiac autophagy.

Additionally, Trp levels were negatively correlated with autophagy markers and Ki-67, whereas KYNA showed positive correlations with LC3, ATG5, Beclin-1, and Ki-67, suggesting that alterations in KP metabolism may be associated with autophagy and cellular proliferative capacity. Our findings are consistent with previous studies demonstrating the opposing roles of QA and KYNA in the regulation of cellular processes [10]. To our knowledge, direct evidence linking cardiac Trp or KYNA levels with autophagy markers is limited. Therefore, the observed correlations should be interpreted as hypothesis-generating and may suggest a potential interaction between KP metabolism and autophagic regulation that warrants further investigation.

Interestingly, cleaved caspase-3 showed significant positive correlations with both autophagy and proliferation markers, suggesting coordinated regulation of apoptosis, autophagy, and cellular renewal. This observation aligns with accumulating evidence that controlled apoptotic signaling contributes to tissue homeostasis by removing damaged cells and promoting compensatory proliferation of surviving cells [58]. Together with the observed correlations between KP metabolites and autophagy- and proliferation-related markers, these findings suggest that dysregulation of KP metabolism may

contribute to shifts in the balance among autophagy, apoptosis, and cellular proliferation during neuroinflammation-induced cardiac stress.

Although LPS induced marked inflammatory and molecular alterations in cardiac tissue, these changes were not accompanied by significant increases in plasma CK, cTnI, or LDH levels, suggesting that experimental neuroinflammation elicited early myocardial stress without overt cardiomyocyte injury. This observation aligns with the concept that conventional cardiac biomarkers are predominantly released after disruption of cardiomyocyte membrane integrity or the development of necrosis and therefore may remain within the normal range during the early stages of cardiac injury [59]. Accordingly, our findings support previous evidence that conventional circulating biomarkers may not be sufficiently sensitive to detect early or subclinical myocardial alterations and highlight the need for more sensitive molecular indicators of cardiac injury [60].

Furthermore, alongside reductions in inflammatory cytokines and oxidative stress markers, DEX treatment significantly decreased cardiac QA levels, *Bax* expression, and the *Bax/Bcl-2* ratio. These findings align with previous studies demonstrating the anti-inflammatory and anti-apoptotic effects of DEX [61]. Moreover, our results suggest that ICV LPS-induced neuroinflammation shifts cardiac KP metabolism toward QA accumulation, and that DEX may normalize this shift. Our findings are consistent with a previous study showing that DEX modulates IDO expression in organotypic hippocampal slice cultures (OHSCs), suggesting that its anti-inflammatory effects may, at least in part, involve regulation of the KP [14]. However, unlike that study, our results suggest that the reduction in cardiac QA levels after DEX treatment may be more closely linked to modulation of downstream enzymes in the KP than to changes in IDO activity. Because the expression and activity of key KP enzymes were not evaluated in the present study, this interpretation remains speculative. Therefore, further mechanistic studies are needed to elucidate the molecular basis of these alterations, and our findings should be regarded as hypothesis-generating.

Nevertheless, the cardioprotective effects of DEX should be interpreted cautiously. Although DEX attenuated cardiac apoptosis, as evidenced by reduced cleaved caspase-3 levels, plasma cTnI and CK-MB concentrations remained elevated in the LPS+DEX group. This apparent discrepancy between tissue-level molecular changes and circulating cardiac injury biomarkers suggests that attenuation of apoptotic signaling may not fully reflect the overall extent of cardiac injury. Therefore, further studies incorporating histopathological and functional cardiac assessments are needed to clarify the cardioprotective potential of DEX.

Nevertheless, the cardioeffects of DEX should be interpreted cautiously. Although DEX attenuated cardiac apoptotic signaling, as evidenced by reduced cleaved caspase-3 levels, plasma cTnI and CK-MB concentrations remained elevated in the LPS+DEX group. One possible explanation is that suppression of apoptotic signaling may have been accompanied by persistence or compensatory activation of non-apoptotic cell-death pathways, such as necroptosis. However, reduced cleaved caspase-3 levels alone are insufficient to demonstrate a shift from apoptosis to necrotic cell death. Therefore, this interpretation

remains speculative and requires confirmation through histopathological examination and assessment of necroptosis-related markers.

Collectively, these findings provide further support for the existence of a neuroinflammation–heart axis and suggest that dysregulation of KP metabolism and impaired autophagy may contribute to the molecular mechanisms underlying cardiac alterations associated with central neuroinflammation.

Conclusion

Experimental neuroinflammation induced by intracerebroventricular LPS was associated with coordinated inflammatory, oxidative, metabolic, and cellular stress responses in cardiac tissue, characterized by NF- κ B activation, increased pro-inflammatory cytokines, dysregulation of kynurenine pathway metabolism with QA accumulation, impaired autophagy, and altered apoptotic signaling. Dexamethasone attenuated central and peripheral inflammation and reduced QA accumulation but failed to restore autophagy- or proliferation-related markers. However, despite further suppressing apoptotic signaling, the LPS+DEX group exhibited increased circulating cTnI and CK-MB levels. This finding suggests that attenuation of apoptotic signaling alone may not fully reflect the extent of cardiac injury and that alternative cell death mechanisms may also be involved. Correlation analyses further demonstrated close associations between kynurenine pathway metabolites and markers of apoptosis and autophagy, supporting a link between altered KP metabolism and cellular stress responses. Collectively, these findings provide novel evidence for a neuroinflammation–heart axis and suggest that dysregulation of KP metabolism and impaired autophagy may contribute to the molecular mechanisms underlying cardiac alterations during neuroinflammation. Further studies integrating histopathological, functional, and mechanistic analyses are needed to clarify the role of QA accumulation, the biological significance of increased Bcl-2 expression, and whether alterations in cardiac KP metabolism represent adaptive or maladaptive responses to neuroinflammation.

Declarations

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Author contributions

E.A. and A.D.A. designed the study and E.A. performed biochemical and statistical analyses. A.D.A. and E.A. conducted the literature review and interpreted the analysis results.

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The authors declare no competing interests.

Data availability

All data are available upon reasonable request

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Table 6

Table 6 is available in the supplementary files section

Figures

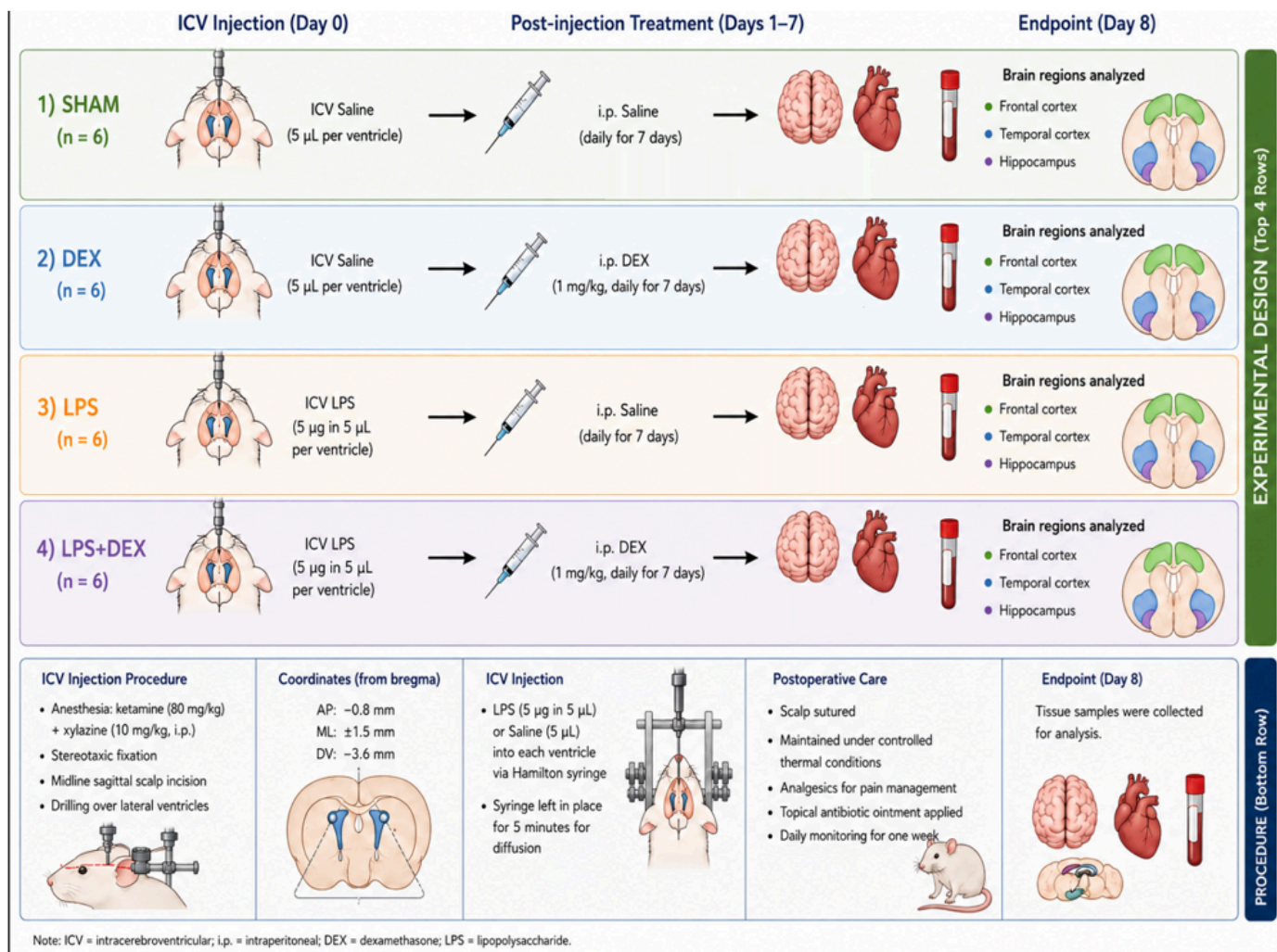


Figure 1

Schematic representation of the experimental design.

Sham, DEX, LPS, and LPS+DEX. On Day 0, animals received bilateral intracerebroventricular (ICV) injections of saline or LPS (5 μ g/5 μ L per ventricle). Subsequently, saline or dexamethasone (DEX; 1 mg/kg, i.p.) was administered daily for 7 days. On Day 8, blood, heart, and brain tissues (frontal cortex, temporal cortex, and hippocampus) were collected for analysis. The lower panel illustrates the ICV injection procedure, stereotaxic coordinates, postoperative care, and tissue collection.

Abbreviations:ICV, intracerebroventricular; LPS, lipopolysaccharide; DEX, dexamethasone; i.p., intraperitoneal; AP, anteroposterior; ML, mediolateral; DV, dorsoventral.

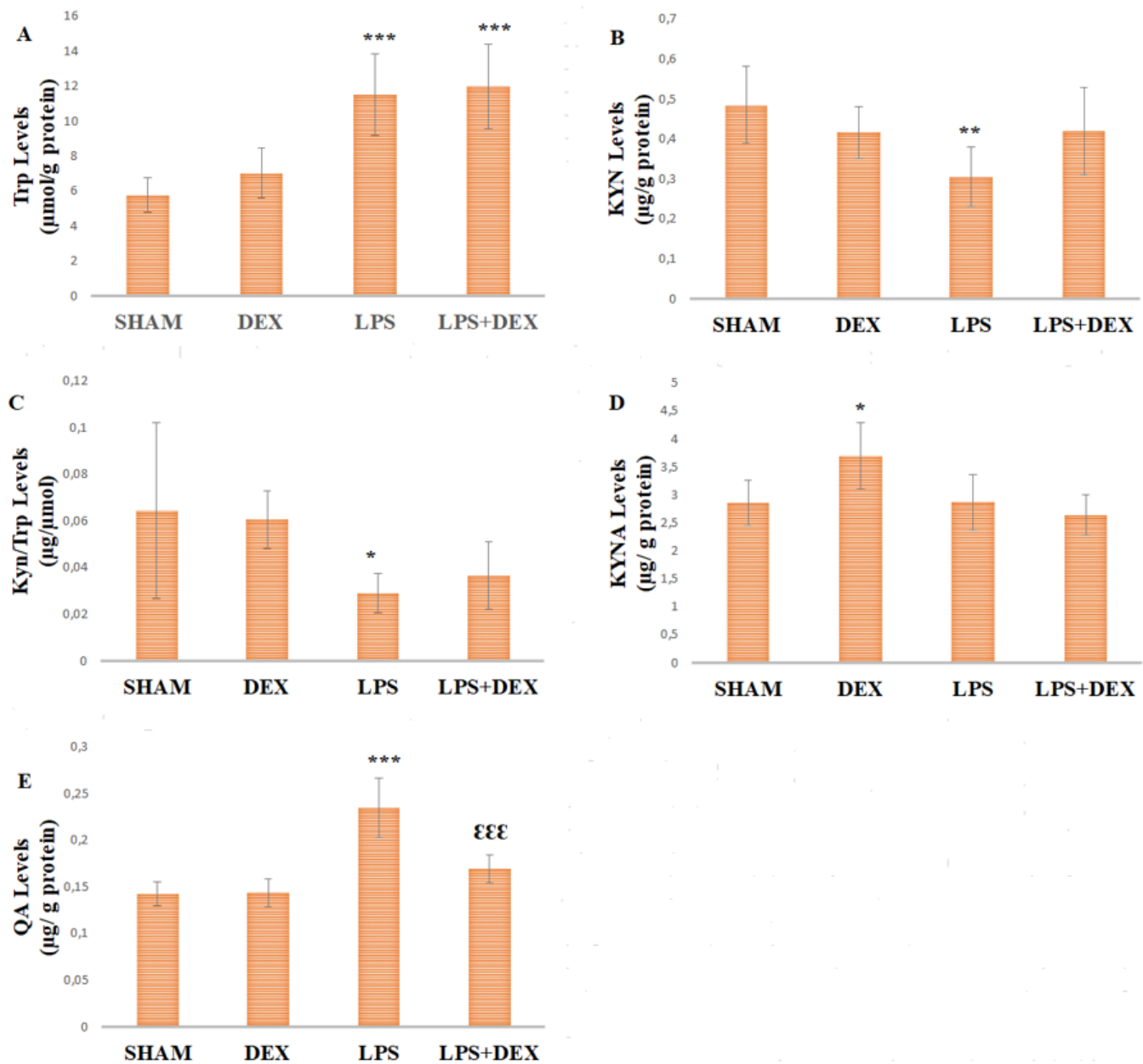


Figure 2

Cardiac kynurenine pathway metabolites in the experimental groups.

(A) Cardiac Trp levels, (B) cardiac Kyn levels, (C) cardiac Kyn/Trp ratio, (D) cardiac KYNA levels, and (E) cardiac QA levels in the experimental groups. Data are presented as mean $\pm$ SD (n = 6 per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *, p<0.05 vs SHAM; **, p<0.01 vs SHAM, *** p< 0.001 vs. SHAM; □□□, p<0.001 vs LPS

Abbreviations: Trp, tryptophan; Kyn, kynurenine; KYNA, kynurenic acid; QA, quinolinic acid.

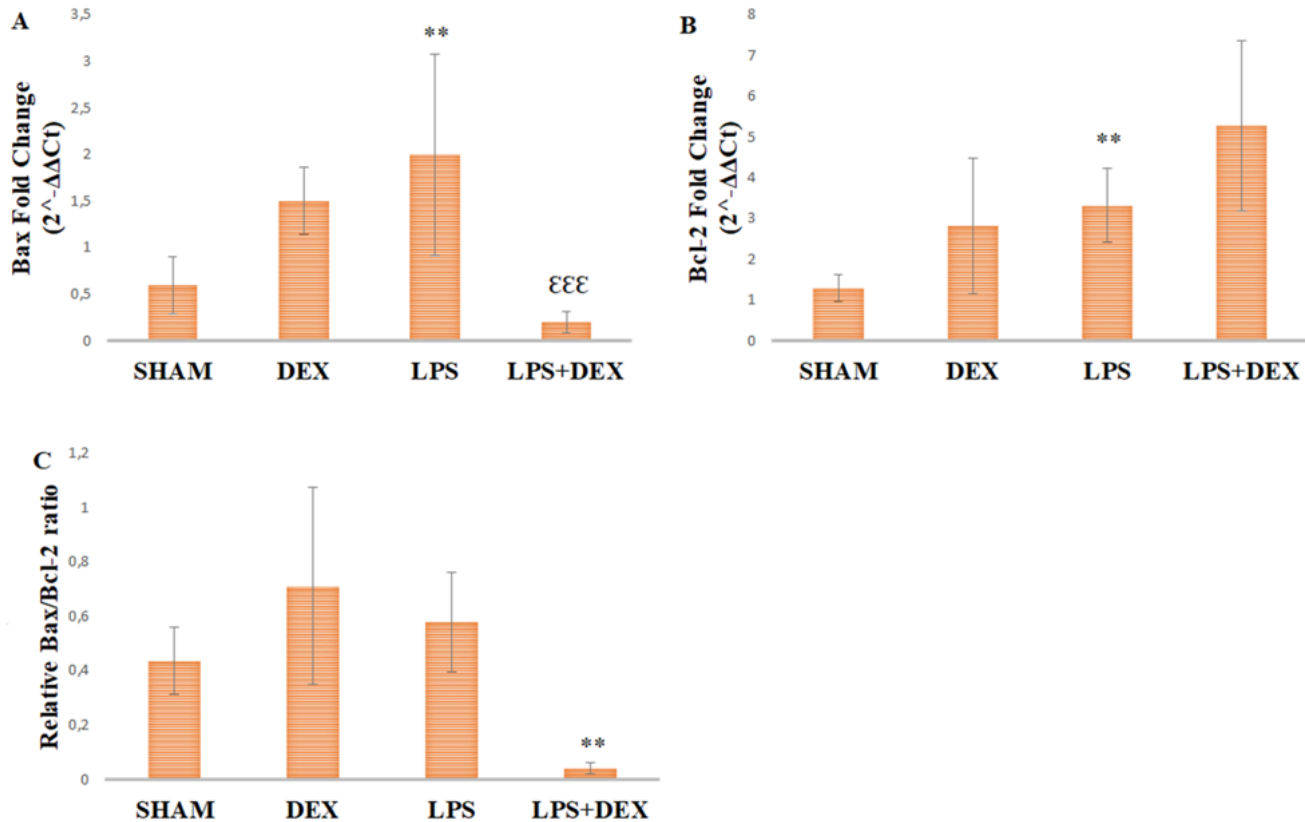


Figure 3

Cardiac *Bax* and *Bcl-2* mRNA expression levels in the experimental groups.

(A) Cardiac *Bax* mRNA expression levels, **(B)** cardiac *Bcl-2* mRNA expression levels in the experimental groups, and **(C)** cardiac *Bax/Bcl-2* ratio in the experimental groups.

Relative *Bax* and *Bcl-2* mRNA expression levels were determined using the $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH. Data are presented as mean $\pm$ SD ($n = 6$ per group). Statistical analysis of *BAX* mRNA expression and the *Bax/Bcl-2* ratio was performed using one-way ANOVA followed by Tukey's post hoc test. For *BCL2* mRNA expression, predefined pairwise comparisons between the SHAM and LPS groups and between the LPS and LPS+DEX groups were performed using the Mann–Whitney U test. * $p < 0.05$ vs. SHAM; ** $p < 0.01$ vs. SHAM; *** $p < 0.001$ vs. LPS.

Abbreviations: *Bax*, *Bcl2*-associated X protein gene; *Bcl-2*, B-cell lymphoma 2 gene; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table6.docx](#)