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Research Article

The Potential Protective Effects of Quercetin Treatment on Hippocampal Tissue of Wistar Rats Exposed to Cadmium

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Abstract

This study aimed to assess the neuroprotective potential of quercetin (Q) against cadmium (Cd)-induced toxicity by evaluating its impact on antioxidant enzyme activities, cytokine profiles, neuronal survival, and histopathological alterations in the hippocampal tissue of rats. Twenty four healthy male Wistar albino rats were randomly assigned into four groups: control (C; n = 6), cadmium (Cd; n = 6), quercetin (Q; n = 6), and cadmium + quercetin (Cd+Q; n = 6). $CdCl_2$ was administered subcutaneously at 4 mg/kg/day for three days to the Cd and Cd+Q groups. Simultaneously, quercetin was administered intraperitoneally at 50 mg/kg/day to the Q and Cd+Q groups. The hippocampal tissues were collected post-treatment for biochemical, histopathological, and cellular analyses. Cadmium exposure led to oxidative stress, elevated pro-inflammatory cytokines, suppressed antioxidant defenses, and neuronal degeneration in the hippocampus. Quercetin treatment partially mitigated these effects by enhancing antioxidant markers (GSH, SOD), modulating cytokine levels (notably IL-1), and preserving neuronal integrity, particularly in the CA1, CA2, CA3, and dentate gyrus regions ($p < 0.05$). These findings suggest that quercetin confers partial neuroprotection against cadmium-induced hippocampal damage through its antioxidative and anti-inflammatory properties. While not entirely preventive, quercetin may serve as a supportive therapeutic agent in countering heavy metal-induced neurotoxicity.

Keywords: Cadmium neurotoxicity, Cytokine modulation, Hippocampal oxidative stress, Neuroprotection, Quercetin

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Introduction

Cadmium (Cd) is an environmentally widespread heavy metal occurring naturally in the Earth's crust. The main natural origins of Cd are associated with the geochemical weathering of metal-containing rocks and volcanic emissions. Additionally, it is commonly found as a minor component in most zinc (Zn) ores and is a byproduct of Zn production (Bouida et al 2022). Furthermore, Cd can be released into the environment from industrial activities such as battery manufacturing, pigment production, stabilizers, and alloy fabrication (Kisadere et al 2021). Cd is well known as a potent environmental pollutant and life-threatening toxic metal. Moreover, Cd is classified as a toxic and carcinogenic heavy metal (Kisadere et al 2022, Zhao et al 2023).

Cd primarily enters the body through oral ingestion (diet, water, vegetables, and plants), inhalation (cigarette smoke), and, less commonly, absorption through the skin (Satarug et al 2010). Once absorbed, Cd exerts harmful effects on various body systems, including the skeletal, central and peripheral nervous, reproductive, cardiovascular, and respiratory systems (Kisadere and Dönmez 2019, Schwartz and Reis 2000, Kisadere et al 2021, Kisadere et al 2022). While the liver and kidneys are recognized as the primary organs for Cd deposition, it may also invade neurons and brain regions (Kisadere et al 2022, Ali et al 2021).

Emerging research indicates that Cd compromises the structural integrity and function of the blood-brain barrier (BBB), facilitating its accumulation within the central nervous system (CNS) (Gupta et al 2016).

Cd buildup in the CNS has been linked to various neurological disorders, including deficits in learning, memory, olfactory function, and behavioral regulation (Sivaprakasam et al 2016). Moreover, several studies suggest a potential association between Cd exposure and the onset of neurodegenerative diseases such as Alzheimer's and Parkinson's (Sivaprakasam et al 2016, Górska et al 2023).

Malondialdehyde (MDA) is a significant secondary product formed during the peroxidation of polyunsaturated fatty acids within various cell types. An increase in MDA levels in circulation is widely recognized as a reliable biomarker of lipid peroxidation (LPO) (Gaweł et al 2004). Several studies have demonstrated that both acute and prolonged exposure to Cd promotes lipid peroxidation processes in central nervous system (CNS) neurons, including oligodendrocytes, microglial cells, and astrocytes (Kisadere et al 2021, Bovio et al 2021, Kisadere et al 2022). The antioxidant defense system, consisting of enzymatic components like superoxide dismutase (SOD) and nonenzymatic antioxidants, including glutathione (GSH), plays a vital role in protecting cells against oxidative stress through the neutralization of free radicals. Experimental data suggest that Cd accumulation in brain tissue results in the overproduction of reactive oxygen species (ROS) accompanied by a marked depletion of key antioxidant defenses, particularly SOD and GSH (Kisadere et al 2022).

In addition, oxidative stress (OS) has been strongly associated with both the excessive production and the dysregulated expression of cytokines involved in inflammatory balance, including interleukin-1 (IL-1), IL-6, IL-10, and tumor necrosis factor-alpha (TNF-α). IL-1 is widely acknowledged as a central pro-inflammatory mediator that influences the regulation of various other cytokines. On the other hand, IL-6, IL-10, and TNF-α, considered anti-inflammatory mediators, are released by immune system cells as a defense mechanism against harmful agents like Cd (Abdalla et al 2014). The harmful impact of both short-term and long-term exposure to Cd on cytokine expression across different brain regions has been highlighted in several experimental investigations (Kisadere et al 2021, Kisadere et al 2022).

Several studies have have indicated that antioxidant compounds such as vitamins E and C, lycopene, selenium, melatonin, chitosan, acetylcysteine, chitosan oligosaccharides, and β-carotene play a significant role in neuroprotection against OS (El-Boshy et al 2015, Kisadere et al 2021). Similarly, Hatipoğlu et al (2023) reported that quercetin effectively alleviated Cd-induced renal dysfunction, while other studies from the same group demonstrated protective effects against Cd -related bone toxicity and oxidative damage (Hatipoğlu et al 2024a, Hatipoğlu et al 2024b). Q (3, 5, 7, 3', 4'-pentahydroxyflavone), a natural antioxidant found in various fruits and vegetables, possesses well-documented antioxidative, anti-inflammatory, antimicrobial, and antiviral properties (Kisadere and Dönmez 2019, Mosolin et al 2022). Notably, Q possesses strong free radical neutralizing capacity and exhibits effective metal-binding properties (Qi et al 2022). Morales et al (2006) demonstrated that Q exerts neuroprotective activity in rat models exposed to Cd-induced neurotoxicity.

The hippocampus, situated deep within the temporal lobe, represents a key anatomical region of the cerebral cortex. It comprises distinct populations of neurons, primarily granule cells (GC) and pyramidal cells (PC), which are structurally organized into layered networks. This brain region is essential for mediating various neurophysiological processes, including spatial orientation, emotional processing, hypothalamic signaling, and the consolidation of episodic and long-standing memories. Furthermore, it constitutes the rear portion of the limbic system, playing a pivotal role in modulating complex behavioral responses (Goncalves et al 2012). According to Kisadere et al (2022), Cd accumulation in this area has been shown to elevate oxidative stress markers and trigger localized inflammatory responses within the hippocampal architecture of rats.

The primary objective of this research was to investigate whether Q administration could exert neuroprotective effects by modulating antioxidant defenses, cytokine profiles, neuronal survival, and histopathological alterations in the hippocampal tissue of rats subjected to Cd exposure.

Material and Methods

Animals

In this experimental study, a total of twenty four healthy, male Wistar albino rats, aged four months and weighing approximately 350 ± 10 g, were utilized. The animals were procured from the Selçuk University Experimental Medicine Research and Application Center (SUDAM), a certified facility specializing in laboratory animal breeding and care. Prior to the experimental procedures, all animals underwent a two-week acclimatization period under standardized laboratory conditions to ensure adaptation and minimize stress-related variability.

Following acclimatization, the rats were randomly allocated into four experimental groups to investigate the effects of

cadmium and quercetin treatments: control group (C; n = 6), cadmium group (Cd; n = 6), quercetin group (Q; n = 6), and cadmium plus quercetin group (Cd+Q; n = 6). All animals were maintained under strictly controlled environmental conditions throughout the experimental period, which included housing in plastic cages with free access to standard rodent chow and clean drinking water (~50 mL per rat per day). The laboratory environment was regulated to maintain an ambient temperature of 23 ± 2 °C, relative humidity of 55 ± 10%, and a consistent 12-hour light/dark cycle to ensure physiological stability. Prior to the initiation of the study, the experimental design and procedures were reviewed and authorized by the Selçuk University Experimental Animal Ethics Committee (approval number: 2015/45). All protocols adhered to internationally recognized ethical standards, including the principles of the Helsinki Declaration and institutional guidelines. This research protocol also received confirmation from the Selçuk University Experimental Medicine Research and Application Center Experimental Animals Ethics Committee, dated March 17, 2015, with decision number 2015/45.

After the adaptation period (two-weeks), at a dose of 4 mg/kg/day cadmium chloride ($CdCl_2$) was subcutaneously (s.c.) injected to Cd and also Cd+Q group animals for 3 days. Although cutaneous absorption of Cd is uncommon, subcutaneous (sc) injection is a well-established method for delivering Cd in experimental toxicology studies, ensuring controlled bioavailability and systemic distribution. Previous studies have demonstrated that sc administration of Cd effectively induces systemic toxicity and neuroinflammatory responses, making it a reliable approach for studying Cd-induced neurotoxicity. This method bypasses variability associated with oral administration and provides reproducible exposure levels comparable to environmental Cd accumulation.

Simultaneously, Q was administered intraperitoneally (ip) at a dose of 50 mg/kg/day to the animals in the Q and Cd+Q groups for three consecutive days (Abdel Moneim, 2016). In contrast, the control group (C) did not receive any pharmacological intervention during the experimental period. Both Cd and Q solutions were freshly prepared prior to each administration to ensure stability and effectiveness. Upon completion of the treatment protocols, all animals were humanely euthanized under general anesthesia (thiopental anesthesia, 40mg/kg BW) . Immediately thereafter, hippocampal tissue samples were collected for subsequent biochemical and histopathological evaluations.

Evaluation of cytokine profiles in hippocampal tissue

Upon completion of the experimental protocol, hippocampal tissue samples were carefully and promptly collected from each animal following ethically approved euthanasia procedures. This approach was adopted to ensure the integrity of the tissue samples by preventing autolytic degradation and minimizing postmortem enzymatic activity. Immediately after dissection, the isolated hippocampal tissues were immersed in liquid nitrogen to rapidly halt any residual enzymatic processes and subsequently stored at −80 °C until further biochemical analyses could be conducted.

For cytokine analysis, the frozen tissue samples were mechanically homogenized at 6000 rpm for 10 minutes using an ultrasonic homogenizer (Sigma 18-K, Newtown, Shropshire, UK). Each homogenization was performed with 200 mg of hippocampal tissue suspended in 800 µL of saline solution to achieve optimal extraction efficiency. The homogenates were then centrifuged, and the resulting supernatants were used for the quantification of cytokine levels.

The concentrations of key cytokines, specifically interleukin-1 (IL-1), IL-6, IL-10, and tumor necrosis factor-alpha (TNF-α), were determined using the enzyme-linked immunosorbent assay (ELISA) technique. These measurements were conducted with the ELX-800 microplate reader system (Biotech Winooski, VT), employing high-quality reagent kits sourced from ThermoFisher (San Jose, CA) to ensure assay reliability and reproducibility.

Assessment of lipid peroxidation and antioxidant status in hippocampal tissue

Lipid peroxidation levels, represented by malondialdehyde (MDA) concentrations, along with glutathione (GSH) amounts and superoxide dismutase (SOD) enzyme activities, were analyzed in the hippocampal tissue supernatants. These biochemical parameters were determined using the ELISA method on the ELX-800 platform (Biotech, Winooski, VT), utilizing commercial assay kits provided by Cayman Chemical (Ann Arbor, MI).

Histopathological examination

Following tissue collection, the rat brain samples were fixed in 10% neutral buffered formalin for a period of one week to ensure optimal preservation of morphological structures. After fixation, standard histological processing procedures were applied, including dehydration through graded alcohol series, clearing, and paraffin embedding. Subsequently, paraffin blocks containing hippocampal sections were prepared. Serial tissue sections, with a thickness of 5–6 µm, were obtained using a microtome and specifically selected to encompass the hippocampal regions.

The sections were mounted onto glass slides and subjected to Crosmann's triple staining technique, as described by Culling et al (1985), to enhance visualization of

cellular components and tissue architecture. The stained slides were examined using a light microscope (Leica DM2500, Switzerland) under appropriate magnification. Comprehensive histopathological assessments were performed to evaluate morphological alterations, including cellular degeneration, nuclear changes, and structural integrity. Additionally, high-resolution images of the relevant hippocampal areas were captured using a digital imaging system (Leica DFC320, Switzerland) for documentation and analysis.

Determination of cell numbers in different parts of the hippocampal tissue

Cell count of GC in the dentate gyrus and PC in the Cornu ammonis (CA) regions, including CA1, CA2, and CA3, were measured. Cell count was performed by using the LAS (Leica Application Suit, Leica Microsystems, Switzerland). It was done on an area of 15000 μm^2 (0,015 mm^2) for the dentate gyrus and the CA1, CA2, and CA3 fields in all the studied groups.

Statistical analysis

In this study, One-Way ANOVA was utilized to analyze the differences among independent experimental groups (Control, Cd, Q, and Cd+Q). This statistical approach was selected as the primary objective was to assess the main effects of Cd exposure and Q treatment on biochemical and histopathological parameters. Since the study design did not incorporate factorial interactions between multiple independent variables, the application of Two-Way ANOVA was not deemed appropriate.

One-Way ANOVA is a widely accepted method in toxicological research when comparing treatment effects across multiple groups. Furthermore, the use of post-hoc testing (Duncan's test) following ANOVA enabled detailed pairwise comparisons, ensuring a robust evaluation of treatment-specific effects rather than interaction effects.

Statistical evaluations were conducted using SPSS software, version 25.0 (SPSS Inc., Chicago, IL). The significance of differences between experimental groups was determined through the application of One-Way Analysis of Variance (ANOVA), followed by Duncan's post-hoc test to perform pairwise comparisons. A p-value of ≤ 0.05 was considered the threshold for statistical significance in all analyses.

Results

Hippocampal tissue MDA concentrations and some antioxidant levels

Cd treatment decreased the hippocampal tissue MDA concentrations in the Cd and Cd+Q groups compared to the C group ($p < 0.05$). In addition, tissue SOD enzyme activity and also GSH values were negatively affected by the Cd administrations in the Cd and Cd+Q group animals when compared to other experimental groups ($p < 0.05$), shown in Table 1.

Hippocampal tissue some cytokine levels

The IL-1 concentrations were observed to be significantly elevated in the Cd group when compared to the C, Q, and Cd+Q groups ($p < 0.05$). Nevertheless, administration of Q resulted in a partial reduction of IL-1 levels within the Cd+Q group relative to the Cd group. Additionally, the levels of IL-6 and TNF-α in hippocampal tissue were found to be substantially higher in both the Cd and Cd+Q groups compared to the Q and C groups ($p < 0.05$). Regarding IL-10, its concentration was recorded as the lowest in the Cd group, while a tendency toward reduction was also noted in the Cd+Q group relative to the Cd group, as illustrated in Table 2.

Histopathological findings

The histological assessment of hippocampal tissue revealed that the control (C) group exhibited normal cellular architecture without any detectable pathological alterations. Similarly, the quercetin-treated (Q) group demonstrated preserved histomorphological features, comparable to those observed in the control group. In contrast, animals exposed solely to Cd displayed pronounced multifocal histopathological changes within the hippocampal region. Detailed microscopic

Table 1. The comparision of tissue MDA and some antioxidant levels in experimental groups (X ± SEM, n = 6).

Parameters	C	Q	Cd	Cd+Q	p
MDA (nmol/mg protein)	0.56 ± 0.07^{b}	0.63 ± 0.06^{b}	1.10 ± 0.08^{a}	0.98 ± 0.13^{a}	0.000
GSH (μM/mg protein)	2.75 ± 0.66^{a}	2.15 ± 0.55^{a}	0.53 ± 0.08^{b}	0.85 ± 0.44^{b}	0.002
SOD (U/mg protein)	0.82 ± 0.06^{a}	0.79 ± 0.03^{a}	0.56 ± 0.43^{b}	0.62 ± 0.03^{b}	0.004

[a,b,c] p: The differences between average values indicated by different letters in the same row for the same parameter are significant ($p < 0.05$). C: Control; Cd: Cadmium; Q: Quercetin; Cd+Q: Cadmium + Quercetin.

Table 2. The comparison of hippocampal tissue cytokine levels among experimental groups (X ± SEM; n = 6).

Parameters	C	Q	Cd	Cd+Q	p
IL-1 (pg/mg protein)	13.14 ± 1.39^{b}	14.02 ± 1.29^{ab}	21.96 ± 2.74^{a}	19.53 ± 1.84^{ab}	0.023
IL-6 (pg/mg protein)	37.08 ± 2.07^{b}	35.80 ± 3.16^{b}	61.67 ± 6.89^{a}	55.85 ± 5.55^{a}	0.001
IL-10 (pg/mg protein)	48.63 ± 5.30^{a}	49.64 ± 6.11^{a}	33.44 ± 3.20^{b}	39.43 ± 3.28^{ab}	0.002
TNF-α (pg/mg protein)	1.41 ± 0.47^{b}	1.33 ± 0.45^{b}	5.41 ± 1.14^{a}	4.56 ± 0.59^{a}	0.000

^{a,b,c} p: The differences between average values indicated by different letters in the same row for the same parameter are significant ($p < 0.05$). C: Control; Cd: Cadmium; Q: Quercetin; Cd+Q: Cadmium + Quercetin.

evaluations in this group identified significant neuronal degeneration, characterized by shrunken pyramidal cells (PC) and granule cells (GC), the presence of pericellular halos, and nuclei exhibiting intense staining accompanied by nucleolar loss. In the group receiving combined Cd and quercetin treatment (Cd+Q), although certain granule cells (GC) exhibited marked signs of degeneration, a proportion of cells maintained typical histomorphological characteristics (Figure 1).

Cell counts

Results about the cell count in different regions of the hippocampal tissue were given in Table 3.

Discussion

Recent researches have shown that extreme free radical production in the different parts of the brain, especially hippocampal tissue, and the imbalance between sources of OS and antioxidant defense systems are associated with the pathogenesis of neuro-degenerative disorders (Özmen et al 2014, Kisadere et al 2022).

In the present study, administration of Cd was found to elevate lipid peroxidation (LPO) activity, as evidenced by increased malondialdehyde (MDA) concentrations in hippocampal tissue, particularly within the Cd and Cd+Q groups when compared to the control (C) group. These findings align with the observations of Kanter et al (2016), who reported that chronic Cd exposure significantly raised hippocampal MDA levels as well as protein carbonyl (PC) content relative to controls. In agreement with our findings, Hatipoğlu et al (2023) observed that Q mitigated cadmium-induced oxidative stress in renal tissues. Additional studies by the same group highlighted antioxidative and anti-inflammatory effects of various agents against cadmium-induced bone toxicity (Hatipoğlu et al 2024a, Hatipoğlu et al 2024b), supporting the relevance of antioxidant-based strategies in cadmium-related tissue damage. Similarly, Kisadere et al (2022) documented elevated MDA concentrations in rat hippocampal tissue following chronic Cd treatment, further supporting the oxidative stress-inducing potential of Cd. These results are consistent with other studies emphasizing the oxidative impact of Cd on neural tissues (Oboh et al 2020, Kisadere et al 2021, Ojo et al 2023). Based on these observations, it may be concluded that the Cd dosage applied in our experimental design contributed to the enhancement of lipid peroxidation processes in the hippocampus.

In contrast, the Q treatment administered in the Cd+Q group did not lead to statistically significant changes in MDA concentrations when compared to the Cd alone group under the conditions of this study. However, this outcome differs from the findings of Kanter et al (2016), who demonstrated that Q supplementation could

Table 3. The count of neurons in different regions of the hippocampal tissue among experimental groups (X ± SEM; n = 6).

Parameters	C	Q	Cd	Cd+Q	p
CA1	33.16 ± 0.87^{a}	34.22 ± 1.03^{a}	24.88 ± 0.88^{c}	29.88 ± 1.42^{b}	0.000
CA2	20.16 ± 0.89^{ab}	22.44 ± 1.40^{a}	15.66 ± 0.55^{c}	19.33 ± 0.88^{b}	0.001
CA3	14.22 ± 0.69^{ab}	16.61 ± 1.13^{a}	11.72 ± 0.43^{c}	13.27 ± 0.51^{b}	0.003
Dentate gyrus	78.72 ± 3.05^{a}	80.33 ± 2.12^{a}	60.05 ± 1.33^{b}	75.77 ± 2.06^{a}	0.002

^{a,b,c} p: The differences between average values indicated by different letters in the same row for the same parameter are significant ($p < 0.05$). CA: Cornu ammoni. C: Control; Cd: Cadmium; Q: Quercetin; Cd+Q: Cadmium + Quercetin.

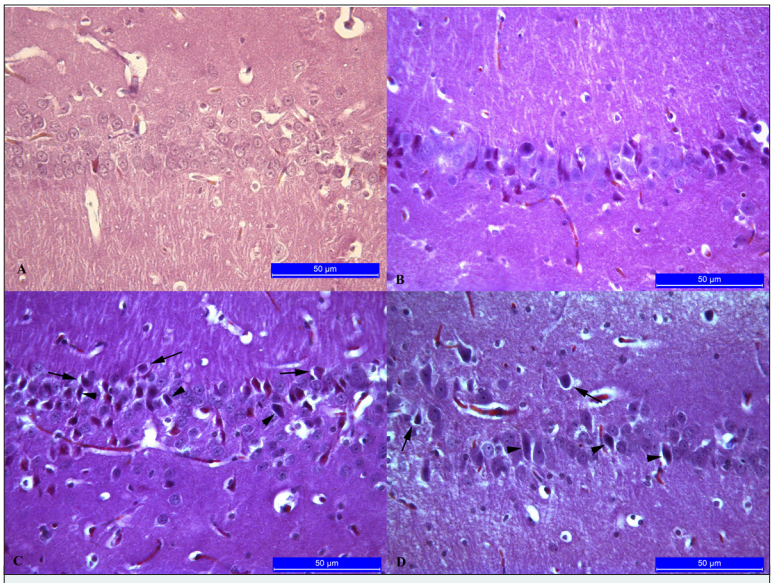

Figure 1. Microscopic view of the hippocampus in Wistar rats from different experimental groups (Crosman's triple staining). A: Control group (C), B: Quercetin-treated group (Q), C: Cadmium-treated group (Cd), D: Cadmium and quercetin-treated group (Cd+Q). Arrowhead: Shrunken cells; Arrow: Pericellular halos. Scale bar: 50 µm.

mitigate Cd-induced elevations in hippocampal MDA levels. Supporting this, Alshammari et al (2021) also reported that Q administration effectively reduced MDA accumulation in Cd-exposed rats. Protective effects of Q on oxidative stress parameters in hippocampal tissue have been observed across both in vivo and in vitro models, as noted in previous research (Wang et al 2022, Wróbel-Biedrawa et al 2022). Such discrepancies across studies may be attributable to differences in experimental protocols, including variations in dosing regimens and exposure durations.

In the present study, hippocampal tissue SOD enzyme activity and also GSH values were adversely affected by the Cd treatments. Treviño et al (2022) informed that Cd exposure causes a severe oxidative response that contributes critically to hippocampal neuro-degeneration. Kisadere et al (2022) proposed that chronic Cd treatment led to a decrease in hippocampal tissue catalase (CAT), SOD enzyme activities, and also GSH values in Wistar rats. Chouit et al (2021) also reported that antioxidant enzyme activities (GSH and CAT) in the striatum and hippocampal tissue were lower in the Cd treatment group compared with the C group. It can be concluded that previous studies support our findings. On the other hand, Q treatment did not statistically alter hippocampal tissue SOD activity and GSH values, however it interestingly tended to increase in our short-term study. Abdalla et al (2014) suggested that Q prevented the reduction in total thiols (T-SH), GSH and glutathione reductase (GR) activities, and the rise of glutathione S-transferases (GS-T) activity in the CNS of the rats. These results were consistent with the previous studies (Xia et al 2015, Kanter et al 2016, Xu et al 2019). Thus, it can be offered that Q might inhibit Cd-induced hippocampal tissue damage (Kanter et al 2016).

Inflammation serves as a key defensive mechanism in response to tissue injury or infection, with cytokines functioning as central mediators in the regulation of this process (Das and Balakrishman 2011). Among these cytokines, interleukin-1 (IL-1) plays a prominent pro-inflammatory role. In the present study, IL-1 levels were significantly elevated in hippocampal tissues of rats exposed to Cd when compared to the control group. This observation is consistent with previous findings by Ali et al (2021), who reported a marked increase in IL-1-beta (IL-1β) expression in the rat brain cortex following Cd exposure. Similarly, Khan et al (2019) demonstrated enhanced IL-1β immunoreactivity in the hippocampus under Cd treatment.

Q administration, on the other hand, induced a modest reduction in IL-1 levels within the hippocampal tissue of Cd+Q group animals in this study. These results are in line with the report by Gupta et al (2016), suggesting that Q mitigates IL-1β elevation in Cd-induced neurotoxicity. Despite these findings, information regarding the influence of Q on IL-1 regulation in rat hippocampal tissue remains limited in the current literature.

Beyond IL-1, other pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), were also measured at significantly higher levels in the Cd group compared to controls. These outcomes corroborate the earlier research by Kisadere et

al (2021), which documented similar cytokine alterations following Cd exposure. However, Q treatment did not significantly affect hippocampal IL-6 and TNF-α levels in the Cd+Q group relative to the Cd-only group in the present investigation. In contrast, Alshammari et al (2021) reported that Q supplementation improved these cytokine profiles in Cd-exposed rats. Other studies have also highlighted the immunomodulatory potential of Q in different regions of the central nervous system (Jung et al 2010, Gupta et al 2016, Wang et al 2022, Olayinka et al 2022). Taken together, these findings suggest that the cytokine-modulating capacity of Q may vary depending on factors such as tissue type, dosage, and treatment duration.

Histopathological changes in the cerebral cortex and hippocampal tissue caused by Cd toxicity have been revealed in previous studies (Kisadere et al 2021, Kisadere et al 2022). Alese et al (2018) reported that Cd treatment caused distortion in the PS of the rat hippocampus. Especially, in the CA3 region, cells with pyknotic nuclei and vacuolization. Montes et al (2015) also reported that Cd treatment led to mild interstitial edema and a few pyknotic cells in the cerebellum and hippocampal tissue of the developing rats. Kanter et al (2016) suggested that significant degenerations, shrunken cytoplasm, and cells with pyknotic nuclei in neurons in the hippocampus were observed in rats exposed to Cd. Mehany et al (2022) also revealed that Cd administration caused characteristic vascular changes associated with diffuse neuronal degenerations. In this study, severe multifocal degenerations, shrinkage of GC, pericellular halos, and cells with heterochromatic nuclei were observed in the hippocampal tissue. These findings were similar with the above-mentioned previous studies. In this study, some of the GC in the hippocampal tissue of rats in the Cd+Q group showed signs of cellular degeneration, while some of them showed normal histomorphological features. Kanter et al (2016) demonstrated that the findings observed in Cd exposed rat's hippocampus were reduced by using Q treatment in rats. Mehany et al (2022) reported that the application of Q caused the hippocampus to appear in its known normal structure. It may be offered that Q might inhibit Cd-induced brain damage in rats. Al-Nouri et al (2025) demonstrated that the neuroprotective effects of Q are mediated through PPARγ activation. The findings obtained in this study support the results of Al-Nouri et al (2025).

The different types of neuronal cells are neatly organized into layers in the hippocampal tissue. In our study, we also demonstrated the effects of Cd and Q on the cell (neuron) counts in the dentate gyrus and the cornu ammonis (CA) (CA1, CA2, and CA3) fields of the hippocampus. A significant decrease of the cell counts in CA1, CA2, CA3, and dentate gyrus regions was observed in the Cd group in the present study. Similarly, Amer et al (2020) suggested that there was a significant decrease in the count of cells in CA1, CA3, and dentate gyrus of rats exposed to Cd. In addition, a significant neuronal loss in both all cornu ammonis regions (CA1, CA2, and CA3) and dentat gyrus areas were detected by Wang et al (2008). Daniel et al (2004) also found similar results according to cell loss in the rat hippocampal tissue. On the other hand, Q treatment ameliorated the Cd-induced cell loss in CA1, CA2, CA3, and dentat gyrus regions in the Cd+Q group compared to Cd group in our study. Daniel et al (2004) suggested that curcumin treatment ameliorated the loss of neurons in the CA1 and CA3 areas of the rats treated with lead (Pb). In a previous study, it was informed that Cd treatment led to damage in principle cells (PrC) of dentate gyrus, and also CA3 and CA1 fields of the rat hippocampus. Conversely, L-carnitine administration reduced the Cd-induced neuronal damage in the same study (Amer et al 2020). We could not find any information about the effects of Q treatment on different cell counts in the hippocampal tissue of rats exposed to Cd in the literature. It can be described as Q has potential protective effects in rat hippocampal tissue.

Conclusion

In conclusion, our results show that cadmium exposure induces significant oxidative stress, disrupts cytokine balance, and causes neuronal loss along with histopathological damage in the hippocampal tissue of rats. These observations confirm the neurotoxic potential of cadmium, particularly its role in promoting inflammation and impairing antioxidant defense mechanisms.

Moreover, our findings indicate that Q treatment offers partial neuroprotection against cadmium-induced hippocampal injury. Specifically, Q contributed to the enhancement of antioxidant enzyme activities and glutathione levels while regulating pro- and anti-inflammatory cytokines, notably by reducing IL-1 levels. In addition, Q was found to improve neuronal survival in critical hippocampal subregions, including CA1, CA2, CA3, and the dentate gyrus, and mitigated cadmium-induced histopathological alterations.

Although the protective effect of Q was not absolute, its ability to modulate oxidative stress and inflammatory responses underscores its therapeutic potential as a supportive agent against heavy metal-induced neurotoxicity. Further investigations, particularly focusing on long-term exposure models and optimal dosing regimens, are necessary to fully clarify its mechanisms of action and efficacy.

These results contribute valuable evidence to the

understanding of natural antioxidant-based interventions in environmental neurotoxicology and suggest that Q could be considered as a promising adjunctive strategy for reducing cadmium-related neural damage.

Declarations

Competing Interests
Authors declare that there are no conflicts of interest related to the publication of this article.
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Availability of Data and Materials
The data that support the findings of this study are available on request from the corresponding author.

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Ethical Statement
All experimental procedures involving animals were approved by the Selcuk University Experimental Animal Ethics Committee (approval number: 2015/45).

Author Contributions
Motivation/Concept: MO, IK, HHD, ND, IU; Design: MO, IK, HHD, ND, IU; Control/Supervision: MO, IK, HHD, ND, IU; Data Collection and Processing: MO, IK, HHD, ND, IU; Analysis and Interpretation: MO, IK, HHD, ND, IU; Literature Review: MO, IK, HHD, ND, IU; Writing the Article: MO, IK, HHD, ND, IU; Critical Review: MO, IK, HHD, ND, IU

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