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Evaluation of the Effects of Chrysin in Rats Developed with Lead Acetate-Induced Neurotoxicity Using Biochemical, Histopathological, and Immunohistochemical Methods

Özge KANDEMİR [1, a]
Elif DALKILINÇ [2, b]
Aybüke Gözde GÜLTEKİN UÇAR [3, c]
Fulya BENZER [4, d]
Fatih Mehmet KANDEMİR [5, e]
Serkan YILDIRIM [6, 7, f]
Sefa KÜÇÜKLER [2, g]

[1] Aksaray University, Aksaray Vocational School of Technical Sciences, Department of Food Processing, Aksaray, TÜRKİYE

[2] Atatürk University, Faculty of Veterinary Medicine, Department of Biochemistry, Erzurum, TÜRKİYE

[3] Atatürk University, Faculty of Medicine, Department of Public Health, Erzurum, TÜRKİYE

[4] Munzur University, Faculty of Health Sciences, Department of Midwifery, Tunceli, TÜRKİYE

[5] Aksaray University, Faculty of Medicine, Department of Medical Biochemistry, Aksaray, TÜRKİYE

[6] Atatürk University, Faculty of Veterinary Medicine, Department of Pathology, Erzurum, TÜRKİYE

[7] Kyrgyz-Turkish Manas University, Faculty of Veterinary Medicine, Department of Pathology, Bishkek, KYRGYZSTAN

[a] ORCID: 0000-0001-8884-4168
[b] ORCID: 0009-0005-1008-111X
[c] ORCID: 0009-0000-0928-1134
[d] ORCID: 0000-0003-1988-4267
[e] ORCID: 0000-0002-8490-2479
[f] ORCID: 0000-0003-2457-3367
[g] ORCID: 0000-0002-8222-5515

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Correspondence
Özge KANDEMİR
Aksaray University, Vocational School of Technical Sciences, Department of Food Processing, Aksaray – TÜRKİYE
kandemirozge23@gmail.com

Lead (Pb) is a heavy metal with high toxicity that can cross the blood-brain barrier and accumulate in brain tissue, causing serious neurological disorders. The aim of this study is to evaluate the potential protective effect of chrysin (CHR) against Pb-induced neurotoxicity. Thirty-five male Sprague Dawley rats were divided into five groups: Control, CHR (50 mg/kg), Pb, Pb+CHR (25 mg/kg), and Pb+CHR (50 mg/kg). Rats were administered CHR (25 or 50 mg/kg) and Pb (30 mg/kg) orally for 7 days. Twenty-four hours after the last treatment, the rats were sacrificed under mild sevoflurane anesthesia by decapitation, and brain tissue samples were collected. Biochemical and histopathological methods were used to measure cholinergic enzyme activity, DNA damage, and oxidative stress parameters in brain tissue. CHR reduced levels of glial fibrillary acidic protein (GFAP), acetylcholinesterase (AChE), and 8-hydroxy-2'-deoxyguanosine (8-OHdG) caused by Pb. Due to its antioxidant properties, CHR decreased Pb-induced lipid peroxidation while increasing glutathione (GSH) levels and the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Based on biochemical and immunohistochemical analyses, CHR reduced cyclooxygenase-2 (COX-2), myeloperoxidase (MPO), nuclear factor kappa-B (NF-κB), neuronal nitric oxide synthase (nNOS), prostaglandin E2 (PGE-2), and p53 levels. These findings suggest that CHR may exert a therapeutic effect by alleviating oxidative stress and inflammation induced by Pb-induced neurotoxicity.

Key Words: *Chrysin, histopathology, immunohistochemistry, inflammation, neurotoxicity, oxidative stress*

Kurşun Asetat Kaynaklı Nörotoksisite Geliştirilen Ratlarda Krisin'in Etkilerinin Biyokimyasal, Histopatolojik ve İmmünohistokimyasal Yöntemlerle Değerlendirilmesi

Kurşun (Pb), kan-beyin bariyerini geçebilen ve beyin dokusunda birikerek ciddi nörolojik bozukluklara yol açabilen yüksek toksisiteye sahip bir ağır metaldir. Bu çalışmanın amacı, krisinin (CHR) Pb'nin neden olduğu nörotoksisiteye karşı potansiyel koruyucu etkisini değerlendirmektir. Otuz beş erkek Sprague Dawley sıçanı beş gruba ayrıldı: Kontrol, CHR (50 mg/kg), Pb, Pb+CHR (25 mg/kg) ve Pb+CHR (50 mg/kg). Sıçanlara 7 gün boyunca oral yolla CHR (25 veya 50 mg/kg) ve Pb (30 mg/kg) uygulanmıştır. Son tedaviden 24 saat sonra sıçanlar hafif sevofluran anestezisi altında dekapitasyon ile sakrifiye edilmiş ve beyin dokusu örnekleri toplanmıştır. Beyin dokusunda kolinerjik enzim aktivitesi, DNA hasarı ve oksidatif stres parametreleri biyokimyasal ve histopatolojik yöntemlerle ölçülmüştür. CHR, Pb'nin neden olduğu glial fibriler asidik protein (GFAP), asetilkolinesteraz (AChE) ve 8-hidroksi-2'-deoksiguanozin (8-OHdG) seviyelerini azaltmıştır. Antioksidan özellikleri sayesinde CHR, Pb'nin neden olduğu lipit peroksidasyonunu azaltırken glutatyon (GSH) seviyelerini ve katalaz (KAT), süperoksit dismutaz (SOD) ile glutatyon peroksidaz (GPx) aktivitelerini artırmıştır. Biyokimyasal ve immünohistokimyasal analizlere göre CHR, siklooksijenaz-2 (COX-2), miyeloperoksidaz (MPO), nükleer faktör kappa-B (NF-κB), nöronal nitrik oksit sentaz (nNOS), prostaglandin E2 (PGE-2) ve p53 seviyelerini azaltmıştır. Bu bulgular, CHR'nin Pb nörotoksisitesinin neden olduğu oksidatif stres ve inflamasyonu hafifleterek terapötik bir etki gösterebileceğini düşündürmektedir.

Anahtar Kelimeler: *Krisin, histopatoloji, immünohistokimya, inflamasyon, nörotoksisite, oksidatif stres*

Introduction

Heavy metals are one of the main causes of environmental pollution (1). Among these heavy metals, lead (Pb) is considered a significant environmental pollutant due to its high toxicity. Pb exposure mostly occurs through contaminated food, water, and air (2). According to the World Health Organization, health problems caused by Pb exposure claim the lives of approximately one million people each year. Pb exposure is estimated to account for approximately 0.6% of the worldwide disease burden. Additionally, it is linked to 4.6% of cardiovascular illness, 3% of chronic kidney disease, and 30% of cases of idiopathic intellectual disability (3).

Pb exposure can affect the central nervous system in children, causing developmental disorders, lower IQ levels, shortened attention spans, hyperactivity, and mental disorders. In adults, this exposure can cause problems including decreased reaction time, memory loss, nausea, insomnia, loss of appetite and joint weakness (4). Pb affects various biological processes such as enzyme inhibition, oxidative stress, and disruption of calcium homeostasis. It has been stated that the neurotoxic effects of Pb occur through oxidative stress by producing reactive oxygen species (ROS), which in turn leads to disruption of neurotransmitter function, lipid peroxidation, inflammation and

cell damage (3). It is also known that ROS levels are increased in neurons of individuals with neurodegenerative diseases, leading to mitochondrial dysfunction, triggering the release of redox metals that interact with oxygen and cause neuronal death (5).

Herbal products containing flavonoids and phenolic compounds are used for therapeutic purposes. Antioxidants protect both humans and animals against infection and degenerative diseases by scavenging free radicals (6, 7). Numerous plants, honey, and propolis contain chrysin (CHR), a naturally occurring flavonoid with significant therapeutic and commercial significance (8, 9). CHR exhibits anti-inflammatory and antitoxic properties due to the absence of oxygenation in the B and C rings, and antioxidant properties due to meta-hydroxylation, double bonds and carbonyl groups. These structural features make CHR valuable for the treatment of liver, neurodegenerative and reproductive disorders (10).

The preventive effect of CHR against brain damage caused by Pb has not been sufficiently documented in the literature. In this study, the possible preventive effects of CHR against brain damage caused by Pb were investigated.

Materials and Methods

Research and Publication Ethics: This study was conducted at Atatürk University Medical Experimental Application and Research Center (ATADEM) following the ethical approval of Atatürk University Experimental Animals Local Ethics Committee dated 30/10/2024 and numbered 2024/10-235.

Chemicals: All chemicals used in the experiment, including Pb (lead (II) acetate trihydrate, Cas no: 6080-56-4) and CHR (Cas no: 480–40-0) were of the highest purity and obtained from Sigma-Aldrich Chemical Company (St Louis, MO, USA).

Animals: The Erzurum Atatürk University Medical Experimental Application and Research Center provided 35 male Sprague Dawley rats, weighing between 250 and 270 grams, for this investigation. The animals were housed in a room with a 12-h light-dark cycle, a relative humidity of 45 ± 5% and a constant temperature of 24-25°C. Before the experiment began, the rats were allowed to acclimate to their surroundings for a week. During the experiment, they were allowed to consume as much water and regular laboratory food as they desired. In the study 35 Sprague-Dawley male rats were split into five groups, each comprising seven rats.

Group I (Control): Rats were given physiological serum via oral gavage for 7 days.

Group II (**CHR):** CHR was administered to rats by oral gavage at of 50 mg/kg for 7 days (11).

Group III (**Pb):** Rats were administered Pb at a dose of 30 mg/kg via oral gavage for seven days (12).

Group IV (Pb + CHR 25 mg/kg): 25 mg/kg CHR was administered orally to the rats 30 minutes after 30 mg/kg Pb administration for seven days.

Group V (Pb + CHR 50 mg/kg): 50 mg/kg CHR was administered orally to the rats 30 minutes after 30 mg/kg Pb administration for seven days. Pb and CHR were dissolved in distilled water, and the resulting suspensions were administered to experimental animals.

Biochemical Analyses: 24 hours after the experimental administrations, the rats were decapitated under mild sevoflurane anesthesia, and brain tissue samples were taken.

To assess oxidative stress markers, brain tissue homogenates were prepared as described in our earlier study (13). The Sedlak & Lindsay (14) method was used to measure glutathione (GSH) levels in brain tissue homogenate supernatants; the Aebi method was used to measure catalase (CAT) activity (15); the Sun et al. (16) method was used to measure superoxide dismutase (SOD) activity; the Lawrence and Burk method was used to measure glutathione peroxidase (GPx) activity (17); the Placer et al. (18) method was used to measure malondialdehyde (MDA) content; and the Lowry et al. (19) method was used to measure total protein.

Determination of AChE Enzyme Activity: Acetylcholinesterase (AChE) enzyme activity was analyzed using a reaction mixture consisting of 50 µL of homogenate, 100 µL of sodium phosphate (0.1 M, pH 8.0), 750 µL of purified water, 50 µL of acetylthiocholine iodide and 50 µL of 5,5′-Dithiobis (2-nitrobenzoic acid) (0.25 mM) according to the method of Ellman et al. (20) and measured with a spectrophotometer (Bio-Tek, Winooski, VT, USA) at a wavelength of 412 nm.

Determination of ELISA Markers: The levels of nuclear factor-kappa B (NF-κB, 201–11-0288), cyclooxygenase-2 (COX-2, 201–11-0297), myeloperoxidase (MPO, 201–11-0575), prostaglandin E2 (PGE-2, 201–11-0505), P53 (201-11-0072), 8-hydroxy-2′-deoxyguanosine (8-OHdG, 201–11-0032) in brain tissue were analyzed by rat ELISA kits according to the manufacturer's procedure (Sunred Biological Technology, Shanghai, China). The absorbance was measured at 450 nm.

Histopathological Analyses: The tissue samples obtained at the end of the evaluation were fixed in a 10% formaldehyde solution for 48 hours, then subjected to routine tissue processing and embedded in paraffin blocks. Sections 4 µm thick were cut from each paraffin block, and the preparations for histopathological examination were stained with hematoxylin-eosin (HE) and examined under a light microscope (Olympus BX51, Japan). The evaluation of neuronal damage was performed by a blinded observer using ImageJ software. The sections were evaluated by a blinded observer using ZEISS Zen Imaging Software according to histopathological features as absent (0), mild (1-2), moderate (3), and severe (4-5).

Immunohistochemical Analyses: Tissue sections mounted on adhesive (poly-L-lysine) slides for immunoperoxidase examination were deparaffinized and dehydrated. Endogenous peroxidase was then inactivated by incubating the sections in 3% H_2O_2 for 10 minutes. The tissues were then boiled in a 1% antigen retrieval solution (citrate buffer (pH+6.1) 100X) and left to cool at room temperature. To prevent nonspecific background staining in the tissues, the sections were incubated with a protein block for 5 minutes. The tissues were then spotted with primary antibody (GFAP Cat No: sc-33673, Dilution Ratio: 1/100, US, nNOS Cat No: ab229785, Dilution Ratio: 1/100, US) and incubated according to the instructions for use. 3-3' Diaminobenzidine (DAB) chromogen was used as the chromogen in the tissues. The stained sections were examined using a light microscope (Zeiss AXIO, Germany).

Statistical Analysis: Biochemical and histopathological analysis data were assessed for normality using the Shapiro–Wilk test, and the data were found to have a normal distribution. To analyze the parametric data, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used, and all data were presented as mean ± standard error (± SEM). GraphPad Prism software (version 8) was used for statistical analysis. To determine positive staining from the images obtained by immunohistochemical staining, five random fields were selected from each image and evaluated using ZEISS ZEN Imaging Software. The data were expressed as the product of area percentage, mean, and standard deviation (mean ± SD). Statistical significance was set at $p<0.05$, $p<0.01$, $p<0.001$, and $p<0.0001$, respectively.

Results

Effect of CHR on Antioxidant Enzymes and Lipid Peroxidation in Pb-induced Brain Toxicity: In lead-induced brain damage, we found that lead increased MDA levels (Figure 1A) compared to the CHR group, while also decreasing GSH levels (Figure 1B) and the activities of antioxidant enzymes such as SOD (Figure 1C), CAT (Figure 1D), and GPx (Figure 1E). On the other hand, CHR25 and CHR50 treatments combined with Pb were found to decrease MDA levels while significantly increasing SOD, CAT and GPx activities and GSH levels compared to the Pb group ($p<0.0001$). Details regarding lipid peroxidation and antioxidant markers in brain tissue are presented in Figure1.

Evaluation of AChE Enzyme Activity: As shown in Figure 2, AChE enzyme activity was increased in the Pb-treated group compared to the CHR group. However, CHR treatment (25 and 50 mg/kg) with Pb significantly ($p<0.0001$) reduced the elevation of AChE activity in a dose-dependent manner. This clearly indicates that CHR significantly inhibits AChE activity and has an anticholinergic effect in the brain.

Evaluation of Inflammation Markers: NF-κB (Figure 3A), MPO (Figure 3B), COX-2 (Figure 3C), and PGE-2 (Figure 3D) levels are summarized in Figure 3. Compared with the control and CHR groups, NF-κB, MPO, COX-2, and PGE-2 levels increased significantly in the Pb group ($p<0.0001$), while they decreased significantly in the Pb+CHR25 and Pb+CHR50 groups compared to the Pb group ($p<0.0001$). CHR alone did not show any significant difference compared to the control group for these parameters ($p>0.05$).

Evaluation of 8-OHdG Level: The level of 8-OHdG, which indicates DNA damage in the brain, is shown in the graph in Figure 4. The Pb-induced group showed a significant increase in brain 8-OHdG levels compared to the CHR group ($p<0.0001$). However, CHR treatment (25 and 50 mg/kg) significantly reduced 8-OHdG levels compared to the Pb group ($p<0.0001$). Nevertheless, no statistically significant difference in 8-OHdG levels was detected between the two different CHR doses administered ($p>0.05$).

Evaluation of p53 Level: P53 levels shown in Figure 5 were higher in the Pb group than in the CHR group, indicating that Pb induced apoptosis in cells ($p<0.0001$). On the other hand, it was determined that Pb protected cells against apoptosis by suppressing p53 expression in CHR treated groups ($p<0.0001$). Nevertheless, no statistically significant difference in P53 levels was detected between the two different CHR doses administered ($p>0.05$).

Histopathological Findings: Examining the brain tissues of the rats in the control and CHR groups revealed that their histological appearance was normal (Figure 6A, B). In the histopathological examination of the brain tissues in the Pb group, severe hyperemia was detected in the brain parenchyma and meningeal vessels, severe degeneration, and necrosis in the neurons in the parenchyma (Figure 6C). In the Pb+CHR25 group, severe hyperemia of the vessels and neuronal degeneration were observed, along with moderate neuronal necrosis (Figure 6D). In the Pb+CHR50 group, mild degeneration in the neurons in the brain tissues and hyperemia in the vessels were observed (Figure 6E).

Immunohistochemical Findings: In this study, GFAP and nNOS expressions were evaluated using immunohistochemical methods, and representative images obtained are presented in Figure 7 and 8. When the brain tissues of the control and CHR groups were examined immunohistochemically, glial fibrillary acidic protein (GFAP) and neuronal nitric oxide synthase (nNOS) expressions were found to be negative (Figure 7A-B, 8A-B). Astrocytes in the Pb group showed severe immunopositivity, while neurons in the brain tissues showed severe GFAP and nNOS expression (Figure 7C, 8C). In the Pb+CHR25 group, moderate GFAP expression was observed in brain tissues and moderate nNOS expression was observed in neurons (Figure 7D, 8D). In the Pb+CHR 50 group, mild GFAP expression was detected in brain tissues, and mild nNOS expression was detected in neurons (Figure 7E, 8E). Figure 9 shows the histopathological scoring (Figure 9A) and the immunohistochemical semi-quantitative scoring results (Figure 9B).

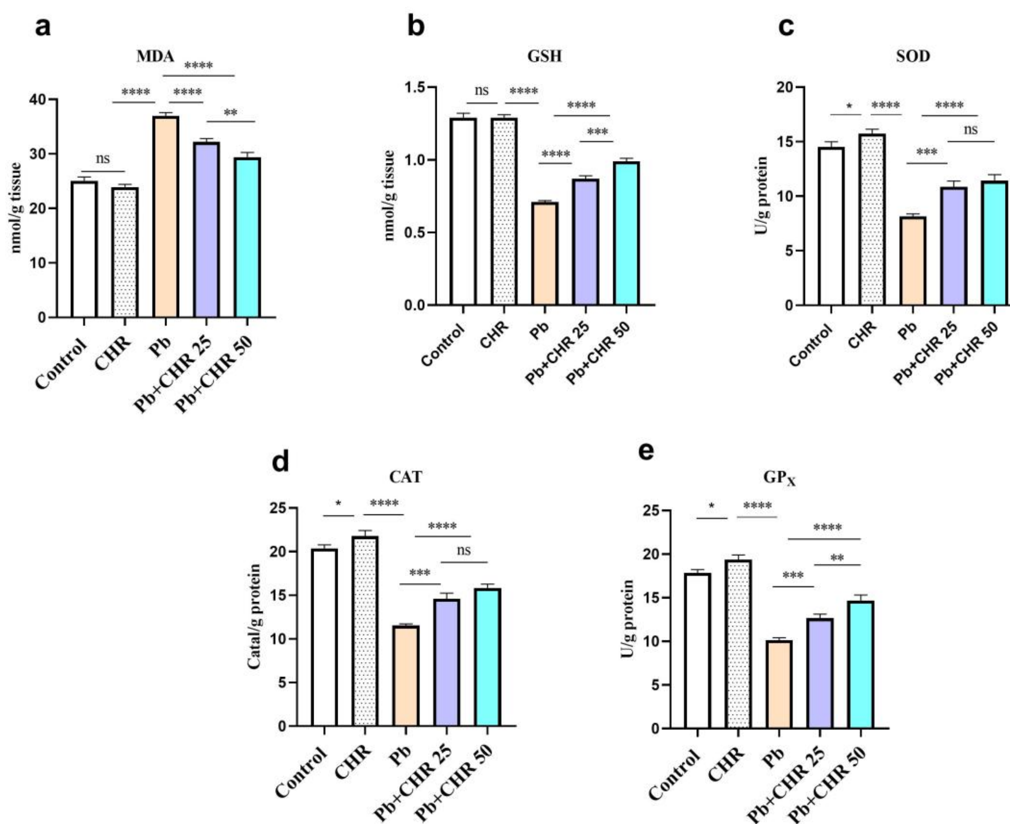

Figure 1. Effects of CHR and Pb administrations on MDA (**a**) and GSH (**b**) levels and SOD (**c**), CAT (**d**) and GPx (**e**) activity in rat brain. MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase; CHR:Chrysin; Pb: Lead. The mean ± SEM is used to express the data. One Way ANOVA was used to examine statistical significance (**p*<0.05, ***p*<0.01, ****p*<0.001, *****p* <0.0001, ns: not significant).

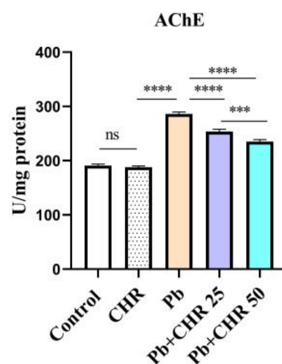

Figure 2. Effect of chrysin on Pb-induced brain AChE activity in rats. AChE: Acetylcholinesterase; CHR: Chrysin; Pb: Lead. The mean ± SEM is used to express the data. One Way ANOVA was used to examine statistical significance (**p*<0.05, ***p* <0.01, ****p*<0.001, *****p*<0.0001, ns: not significant).

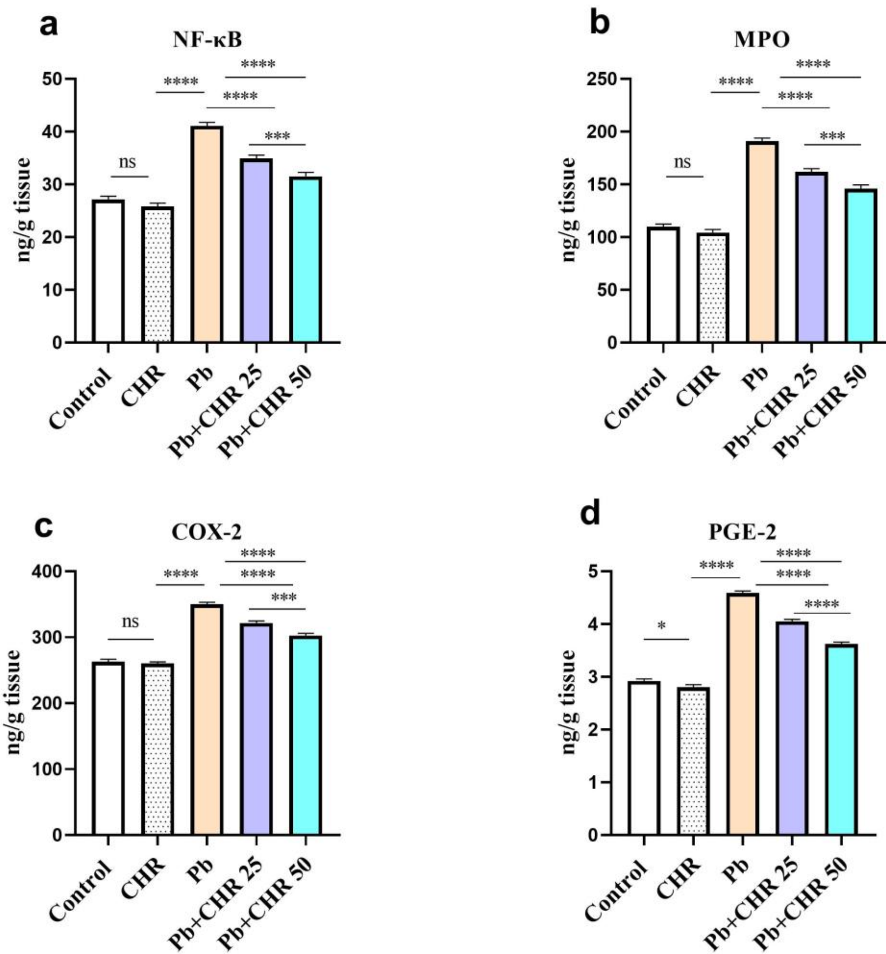

Figure 3. Effect of chrysin on Pb-induced brain NF-κB (**a**), MPO (**b**), COX-2 (**c**), and PGE-2 (**d**) levels in rats. NF-κB: Nuclear factor kappa-B; MPO: Myeloperoxidase; COX-2: Cyclooxygenase-2; PGE2: Prostaglandin-E2; CHR: Chrysin; Pb: Lead. The mean ± SEM is used to express the data. One Way ANOVA was used to examine statistical significance (**p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001, ns: not significant).

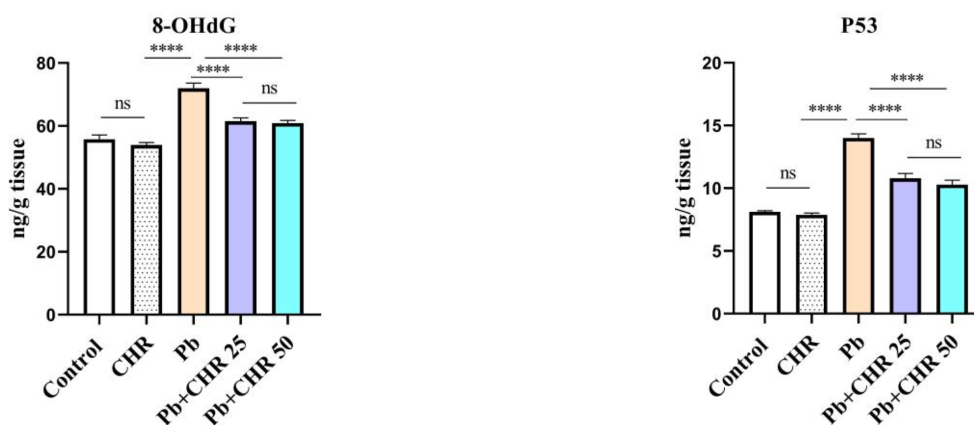

Figure 4. Effect of chrysin on 8-OHdG level in Pb-induced brain injury in rats. 8-OHdG: 8-hydroxy-2′-deoxyguanosine; CHR: Chrysin; Pb: Lead. The mean ± SEM is used to express the data. One Way ANOVA was used to examine statistical significance (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns:not significant).

Figure 5. Effects of CHR on p53 levels in Pb-induced neurotoxicity in rats. CHR: Chrysin; Pb: Lead. The mean ± SEM is used to express the data. One Way ANOVA was used to examine statistical significance (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns: not significant)

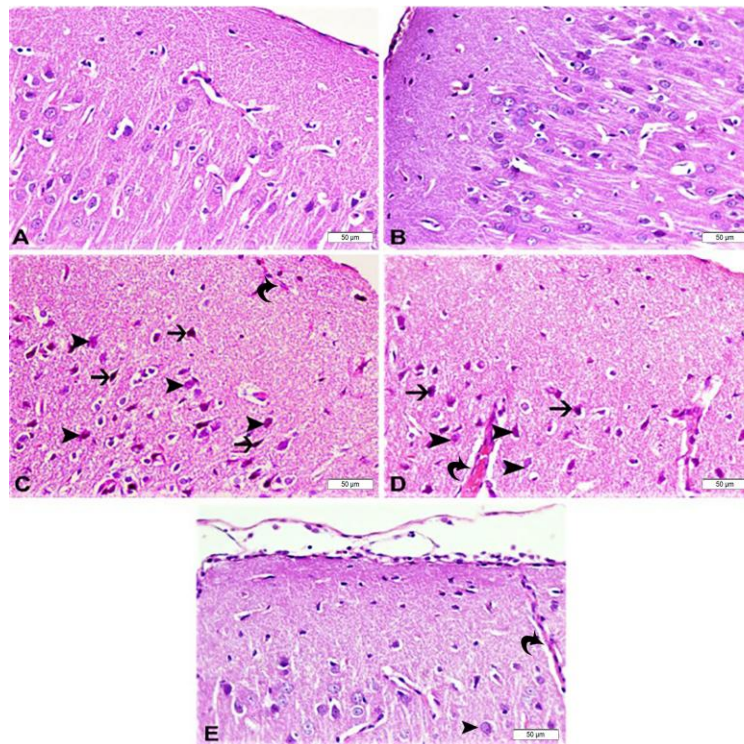

Figure 6. Brain tissue, Control (**A**) and CHR groups (**B**), normal histological appearance, Pb group (**C**), severe degeneration (arrowheads) and necrosis (arrows) in neurons, vascular hyperemia (curve), Pb+CHR25 group (**D**), severe degeneration in neurons (arrowheads), moderate necrosis (arrows), vascular hyperemia (curve), Pb+CHR50 group (**E**), mild degeneration in neurons (arrowhead), vascular hyperemia (curve), H&E, Bar: 50 µm. CHR: Chrysin; Pb: Lead.

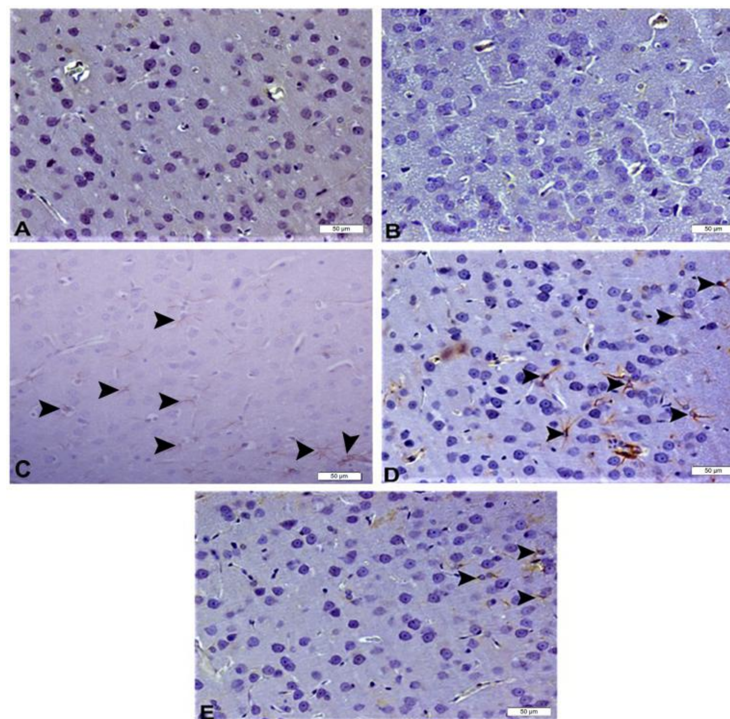

Figure 7. Brain tissue, Control (**A**) and CHR groups (**B**), GFAP expression negative, Pb group (**C**), severe GFAP expression (arrowheads), Pb+CHR25 group (**D**), moderate GFAP expression (arrowheads), Pb+CHR50 group (**E**), mild GFAP expression (arrowheads), IHC-P, Bar: 20μm. GFAP: Glial fibrillary acidic protein; CHR: Chrysin; Pb: Lead.

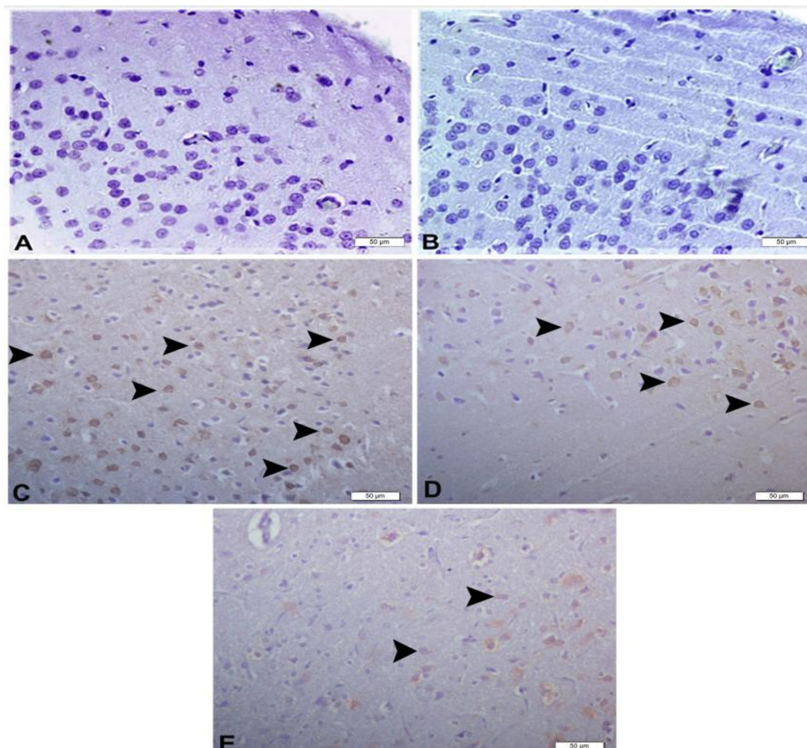

Figure 8. Brain tissue, Control (**A**) and CHR groups (**B**), nNOS expression negative, Pb group (**C**), severe nNOS expression (arrowheads), Pb+CHR25 group (**D**), moderate nNOS expression (arrowheads), Pb+CHR50 group (**E**), mild nNOS expression (arrowheads), IHC-P, Bar: 20µm. nNOS: Neuronal nitric oxide synthase; CHR: Chrysin; Pb: Lead

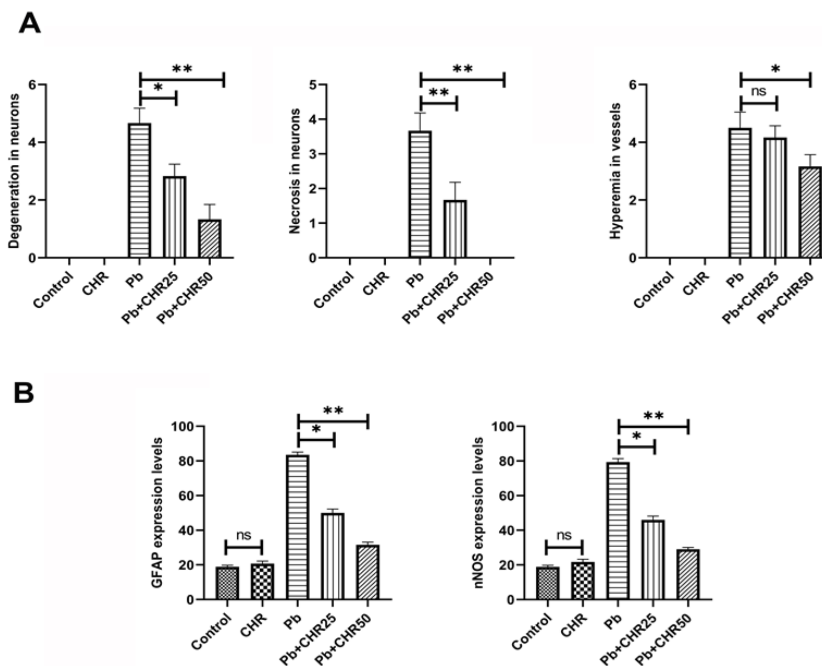

Figure 9. Statistical analysis data for histopathological (**A**) and immunohistochemical (**B**) findings observed in brain tissue. Neuronal degeneration, neuronal necrosis, vascular hyperemia, GFAP expression level, nNOS expression level, (ns= no significant difference, $*p<0.05$, $**p<0.01$). Quantitative expression levels of GFAP and nNOS in brain tissue determined using ZEISS Zen Imaging Software analysis soft ware and related statistical analysis results. Data were evaluated using parametric ANOVA followed by Tukey post hoc test. GFAP: Glial fibrillary acidic protein; nNOS: Neuronal nitric oxide synthase; CHR: Chrysin; Pb: Lead.

Discussion

Pb exposure disrupts brain functions by crossing the blood-brain barrier and poses a danger to human health by causing neurological disorders (21). While interest in dietary supplements containing flavonoids is increasing, positive effects of CHR on plasma membrane functionality, cognitive deficits, lipid peroxidation and inflammation have been demonstrated (10). Our current study showed that CHR played a healing role in brain damage caused by Pb exposure.

The mechanism underlying Pb toxicity is thought to be oxidative stress. Oxidative stress results from the disruption of the balance between ROS and antioxidant defence mechanisms and causes lipid peroxidation, enzymes inactivation, and DNA damage (2, 22). Wang and colleagues suggested that Pb reduces antioxidant enzyme activities by producing free radicals and damages vital organs such as the liver by increasing lipid peroxidation (23). Additionally, Enogieru and Iyoha reported that Pb exposure leads to cerebellar toxicity and neurobehavioral disorders (24). Enzymatic (SOD, CAT, GPx) and non-enzymatic (GSH) antioxidant defense systems play a vital role in protecting cells from this type of oxidative damage (6). Thanks to its antioxidant properties, CHR is a natural flavonoid reported to have neuroprotective effects (25). In a study by Seven et al., CHR ameliorated the adverse effects on liver and kidney tissues caused by Cu toxicity in rats by increasing antioxidant activity and scavenging free radicals (26). The findings obtained in our current study are consistent with these results, and it has been observed that Pb exposure increases MDA levels, a product of lipid peroxidation, while causing a decrease in enzymatic and non-enzymatic antioxidant markers. However, CHR treatment reduced lipid peroxidation in brain tissue due to its antioxidant properties and led to an increase in both enzymatic and non-enzymatic antioxidant markers.

In addition, our study evaluated levels of 8-OHdG, ROS-derivated marker of oxidative DNA damage in cells. As in other studies induced by Pb (27, 28), our data showed that 8-OHdG levels were significantly increased in the Pb-treated group compared to the control and CHR groups. The literature reports that 8-OHdG expression increased by chemotherapeutic drugs, decreases with CHR treatment, thereby reducing in DNA damage (29). Our findings showed that CHR treatment significantly reduced the 8-OHdG levels increased by Pb exposure. These findings emphasize the antioxidant potential of CHR and its preventive effect against cell damage.

The process of programmed cell death known as apoptosis is characterised by specific biochemical and physical changes that cause the cell to shrink and be engulfed by nearby macrophages. Apoptosis in multicellular organisms is essential for both the organism's growth and homeostasis maintenance (30). It has been reported that Pb activates the p53 transcription factor an apoptosis regulator, by increasing oxidative stress (2). Because of the polypeptide's brief half-life, p53 expression is normally maintained at low levels. However, following increased ROS levels and DNA damage, p53 protein levels soon increased significantly (31). In a study by Prajit et al. (25), it was reported that CHR treatment suppressed p53 activity by reducing Bax and caspase-3 expressions and provided protection against neuronal apoptosis by improving the decreased Bcl-2 expression in traumatic brain injury, renal ischemia/reperfusion, and myocardial injury. In this study, it was determined that Pb exposure triggers apoptosis in brain tissue by activating the p53 pathway, but CHR treatment reduces p53 levels by alleviating oxidative stress and DNA damage through its antioxidant properties, thereby providing protection against Pb-related neurotoxicity.

Oxidative stress contributes significantly to the inflammatory process (32). An essential transcription factor, NF-κB controls the expression of genes related to several cellular functions, including immunological response, inflammation, cell division, and survival (12). Under resting conditions, NF-κB is isolated in an inactive form in the cytoplasm and is released when activated by stimuli such as proinflammatory cytokines or oxidative stress (33). Nitric oxide is an essential component of numerous metabolic functions in the central nervous system, when overproduced by nNOS under conditions of neuroinflammation, it increases NF-κB activation, leading to the formation of reactive nitrogen species that can cause neuronal death and damage in the central nervous system (13). The transcription of proinflammatory mediators like tumour necrosis factor (TNF-α), COX-2, interleukin-6, interleukin-1 beta, and inducible nitric oxide synthase is regulated by activated NF-κB (1). Due to its ability to regulate the transcription and activation of proinflammatory mediators, NF-κB has been identified as therapeutically significant. Thus, NF-κB activation inhibition may help prevent brain injury and nervous system diseases (34). MPO is a heme-containing enzyme found especially in neutrophils and to a lesser extent in monocytes (35). MPO has a significant impact on the processes of inflammation and oxidative stress. In neurodegenerative diseases, MPO levels are increased, indicating that the brain is more exposed to oxidative stress and inflammation (36). According to Rehman et al.'s research, CHR significantly reduced NF-κB activation and successfully prevented the rise in ferric nitrilotriacetate-mediated TNF-α, COX-2, and PGE-2 levels (37). Another study using this experimental model of Pb reported that it causes tissue inflammmation by increasing the levels of NF-κB, TNF-α, PGE-2, MPO and interleukin-1 (38). Our findings, as in these studies, show that NF-κB, PGE-2, COX-2, and nNOS levels in brain tissue increase due to Pb-induced oxidative stress, while CHR treatment decreases these levels. Our findings demonstrate that CHR has an anti-inflammatory effect, as previously reported by Küçükler et al. (39). Cholinergic enzymes in neurotransmission, hydrolyse acetylcholine to choline (40). AChE activity was shown to be elevated in a prior investigation involving Pb exposure due to an increase in brain lipid peroxidation and a decrease in antioxidant enzyme activities (41). Increased AChE activity is linked to cholinergic neuron

degeneration (42, 43). Campos et al. reported that CHR treatment attenuated the increased AChE activity in aluminum exposure (44). In our current study, AChE activity was found to be high in the Pb-treated group, while CHR treatment decreased this activity, supporting the neuroprotective potential of CHR.

One of the most commonly used indicators of nerve damage is GFAP, the primary intermediate filament protein expressed in astrocytes of the central nervous system (45, 46). GFAP plays an important role in astrocyte motility, shape, blood-brain barrier integrity, myelination and white matter architecture (47). A previous study reported increased GFAP mRNA levels in rats exposed to Pb (48). Salama et al. showed that CHR reduced GFAP expression (49). In this study, an increase in GFAP expression was observed in the Pb group, while CHR treatment, particularly at a dose of 50 mg/kg, was found to reduce GFAP expression.

In conclusion, our research indicates that CHR has significant promise as a protective agent against Pb-induced neurotoxicity, as evidence by the previously mentioned findings. Thus, CHR's anti-inflammatory, anti-apoptotic, and antioxidant properties in the brain may make it a viable therapeutic agent for a number of neurodegenerative diseases linked to oxidative stress, neuroinflammation, and neuronal apoptosis.

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