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Targeting Neurotoxicity and Inflammation in the Cerebral Cortex with Curcumin-Piperine Synergy

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Abstract

Background: Neuroinflammation, oxidative stress, and demyelination are central features of multiple sclerosis and related neurodegenerative disorders. Cuprizone (CPZ) is widely used to reproduce these pathological changes, inducing profound biochemical and structural alterations in the brain. This study investigated the impact of CPZ-induced neurotoxicity on key antioxidant, inflammatory, cholinergic, and myelin-related markers, and evaluated the therapeutic efficacy of curcumin- and piperine-based nanoformulations designed to enhance neuroprotection and repair.

Method: Male mice received CPZ to induce demyelination and associated oxidative and inflammatory stress. Animals were subsequently treated with a blank nanoformulation (BFZ), curcumin nanoformulation (CFZ), or a combined curcumin–piperine nanoformulation (PFZ). Biochemical analyses quantified antioxidant enzymes (catalase, superoxide dismutase), inflammatory mediators (COX-2, NF-κB-p65), cholinergic activity (acetylcholinesterase), neuroplasticity markers (CREB, NGF), and myelin basic protein (MBP) levels in the cerebral cortex.

Results: CPZ exposure led to marked oxidative stress, neuroinflammation, and neurodegeneration, reflected by decreased antioxidant enzyme levels, elevated COX-2 and NF-κB-p65, impaired cholinergic function, reduced MBP, and suppressed CREB. NGF expression increased significantly in the CPZ group as a compensatory response to neuronal injury. Nanoformulation treatments mitigated these pathological changes, with PFZ demonstrating the strongest therapeutic effect. PFZ restored antioxidant defences, reduced inflammatory markers, enhanced AChE activity, promoted remyelination, and improved CREB expression. PFZ also normalized NGF levels. Across all parameters, PFZ outperformed CFZ and BFZ, highlighting the role of piperine in enhancing curcumin bioavailability.

Conclusion: PFZ exerted potent neuroprotective and reparative effects against CPZ-induced neurotoxicity. These findings emphasize the value of optimized nanoformulations in strengthening phytochemical-based therapies for neurodegenerative diseases.

Introduction

Inflammation is the body's innate response to harmful stimuli, including pathogens, tissue damage, or irritants. It is characterized by the activation of the immune system, with the primary goal of restoring homeostasis and tissue repair. Neuroinflammation refers specifically to the inflammation that occurs within the central nervous system (CNS), which includes the brain and spinal cord. Dysregulation of neuroinflammation is implicated in several neurological conditions, ranging from neurodegenerative diseases to psychiatric disorders [1]. Neuroinflammation is primarily mediated by glial cells, which include microglia, astrocytes, and oligodendrocytes. Microglia are the resident immune cells of the CNS, analogous to macrophages in peripheral tissues. In response to injury, infection, or neurodegeneration, microglia become activated, undergo morphological changes, and release pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and chemokines [2]. While these cytokines are essential for protecting the brain, their overproduction or prolonged activation can contribute to neuronal damage and disease progression in conditions such as Multiple Sclerosis (MS), Alzheimer's disease, Parkinson's disease [3]. Astrocytes, which are the most abundant glial cells in the CNS, respond to inflammation by secreting pro-inflammatory mediators and influencing synaptic function and blood-brain barrier integrity [4]. They play a dual role in neuroinflammation; on one hand, they help modulate immune responses, but on the other hand, chronic activation of astrocytes can exacerbate neuroinflammation, particularly in diseases like MS [5,6]. Neuroinflammation is closely linked to oxidative stress, a state in which reactive oxygen species (ROS) accumulate in the brain. These free radicals are produced by activated microglia, astrocytes, and neurons. Excessive ROS can damage neuronal membranes, proteins, and DNA, contributing to neurodegeneration and neuronal dysfunction [7,8].

Cuprizone is a chemical compound that has been extensively used in experimental research to model neurotoxicity and demyelination in rodents. It induces a reproducible and controlled demyelination process, making it a valuable tool in the study of MS [9]. The cuprizone model has helped elucidate the cellular and molecular mechanisms involved in demyelination, neuroinflammation, and remyelination, providing insights into the pathophysiology of neurodegenerative diseases. Cuprizone toxicity is primarily mediated through its effects on oligodendrocytes, the glial cells responsible for the production and maintenance of myelin in the CNS [10]. Cuprizone induces oxidative stress and mitochondrial dysfunction in oligodendrocytes, which leads to the activation of apoptotic pathways and cell death. This results in the disruption of myelin integrity and the progressive loss of oligodendrocytes in areas such as the corpus callosum and hippocampus leading to demyelination. Cuprizone also induces degeneration of axons, which are normally insulated by myelin. Demyelination of axons impairs the conduction of electrical signals, which may contribute to the neurological deficits as observed in experimental animals [11,12]. One of the most distinctive features of the cuprizone model is the ability of oligodendrocyte precursor cells (OPCs) to undergo differentiation and remyelinate the damaged areas following several days of cuprizone withdrawal. This capacity for remyelination makes the cuprizone model an ideal system for studying the molecular and cellular mechanisms involved in CNS repair and regeneration, and it has been used to explore potential therapeutic approaches to enhance remyelination. The cuprizone model shares many similarities with the pathophysiology of MS, particularly with regard to demyelination and remyelination [13].

Curcumin, a polyphenolic compound derived from the turmeric plant (*Curcuma longa*), and piperine, a bioactive alkaloid found in black pepper, have gained attention for their potential neuroprotective effects [14]. Curcumin is a mixture of three closely related curcuminoids namely curcumin (77%), demethoxycurcumin (17%), and bisdemethoxycurcumin (3%) each contributing to its overall pharmacological activity. Both curcumin and piperine have been shown to modulate neuroinflammation through multiple molecular pathways such as NF-κB Pathway, MAPK (mitogen-activated protein kinase) pathway, antioxidant activity etc making them promising candidates for therapeutic interventions in neurodegenerative diseases [15]. Curcumin has been shown to promote and modulate the activity of microglia, reducing the inflammatory environment. Piperine significantly increases the absorption and bioavailability of curcumin in the body, allowing it to exert more potent effects on neuroinflammation. Without piperine, curcumin has low bioavailability, limiting its therapeutic potential. When combined, piperine facilitates better delivery of curcumin to the brain, enhancing its anti-inflammatory and neuroprotective actions [16].

This study examined the harmful effects of cuprizone on cerebral cortex region of mice brain and the protective role of various curcumin and piperine nanoformulations prepared in *Zanthoxylum rhetsa* seed oil. The assessment focused on changes in antioxidant enzymes/proteins, transcription factors, and cytokines during and after cuprizone intoxication. This study hypothesized that curcumin-piperine nanoformulation (PFZ) would attenuate cuprizone-induced oxidative stress and neuroinflammation through modulation of NF-κB and CREB signaling pathways.

Material and Methods

Animals

Seventy-five Swiss albino male mice (SWR/J) weighing between 21 and 25 grams were acquired from the King Fahd Medical Research Center (KFMRC) animal housing unit at King Abdulaziz University in Jeddah, Saudi Arabia. The male mice were used to reduce hormonal variability. A 12-hour light/dark cycle, with the light cycle occurring between 7:00 am and 7:00 pm, was

implemented to maintain mice at a suitable room temperature of 23±2°C and humidity level of 65%. Food and water were freely available to every mouse. Animal studies were carried out in compliance with KFMRC's animal unit committee rules. The biomedical ethics research committee at King Abdulaziz University authorized the study protocol (approval No. ACUC-22-1-2), which complied with the guidelines established by the KFMRC's Animal Care and Use Committee. The research adhered to the "System of Ethics." The study was authorized by Royal Decree No. M/59 dated August 24, 2010, and it conformed with the "System of Ethics of Research on Living Creatures" rules created by King Abdulaziz City for Science and Technology. From June 1, 2022, until July 16, 2022, the study was conducted at the King Fahad Medical Research Center in Jeddah, Saudi Arabia.

Drug Preparations

The source of cuprizone (CPZ) was Sigma-Aldrich (Bangalore, India). As previously mentioned, mice were given 0.2% w/w CPZ combined with ground rodent chow for five weeks in order to cause acute demyelination [17,18]. By dissolving it separately in *Z. rhetsa* Seed Oil (ZRO) as previously published and defined, a nanoformulation of curcumin with and without piperine was created [19]. To put it briefly, these nanoformulations were created by adding varying ratios of surfactant, cosurfactant, and/or co-solvent to the ZRO. The ZRO was formulated using a fixed amount of cosurfactant (I988) and Tween 85 to examine its effect on formulation performance. The resulting liquid were homogenized and stored until needed. It is basically a self-nanoemulsifying drug delivery system (SNEDDS). For curcumin and piperine, the SNEDDS prepared in ZRO has solubility values as 19.0 mg/g and 48.22 mg/g, respectively. The drug-free ZRO-SNEDDS had a droplet size of 607.1 nm, while the drug-loaded ZRO-SNEDDS had a droplet size of 700.86 nm with curcumin and piperine. The curcumin-piperine loaded SNEDDS (−36.35 mV) and drug-free SNEDDS (−36.9 mV) zeta potentials showed that the resulting emulsion had good physical stability. The dose of curcumin (10 mg/kg) and piperine (3 mg/kg) was selected based on established preclinical studies demonstrating neuroprotection in rodent models [20]. A suitable quantity of piperine, blank, and curcumin with nanoformulation were further diluted in regular saline until 0.1 ml contained 3 mg/kg of piperine and 10 mg/kg of curcumin for each mouse weight. Every day from 11:00 a.m. to 1:00 p.m., 0.1 ml of the formulation was administered intraperitoneally to each mouse. Weekly measurements of the mice's weights were used to modify the dosages.

Experimental Design

The study lasted for seven weeks in total, with demyelination occurring for five of those weeks and remyelination occurring for the final two weeks. A total of fifteen mice each group were randomly assigned to eight primary groups. Control Group: for seven weeks, the control group was given standard chow and 0.1 ml of saline intraperitoneally. Cuprizone group: for five weeks, the cuprizone (CPZ) group got 0.2% CPZ-mixed chow and 0.1 ml of normal saline intraperitoneally. ZRO-SNEDDS Group: this group also received 0.1 ml of ZRO-based blank formulation administered intraperitoneally along with CPZ-mixed chow daily. Curcumin in ZRO-SNEDDS Group: for five weeks, this group was given CPZ-mixed chow and a ZRO-based formulation of curcumin (0.1 ml intraperitoneally). Curcumin-piperine in ZRO-SNEEDS Group: this group received CPZ-mixed chow and a ZRO-based nanoformulation of curcumin with piperine. Cuprizone feeding was discontinued in all groups at the conclusion of the demyelination stage (week 5), but treatment with various nanoformulations continued until the end of week 7. Group names and designations: 1) Control group (CNT); 2) Cuprizone group (CPZ); 3) Blank oil formulation group (BFZ); 4) Curcumin nanoformulation group (CFZ); and 5) Curcumin with piperine nanoformulation group (PFZ). The five-week CPZ regimen induces robust demyelination, followed by a two-week remyelination phase, consistent with standard cuprizone models.

Sample Collection

By the conclusion of the fifth week, a random selection of 7 mice from each group were used to induce isoflurane anaesthesia and decapitate them in order to end their lives. The whole brain of each mouse was then obtained by dissecting it. To get certain brain areas, including the hippocampus, frontal cortex, cerebral cortex, brain stem, and cerebellum, the obtained brains were further dissected. These brain slices were kept for future research at −80°C after being immersed in RNA-later solution. At the conclusion of the study (week 7), the remaining mice underwent the same process.

Biochemical analysis

Tissues from cerebral cortex region were homogenized in RIPA lysis buffer (R-0278; Sigma) containing PMSF at the prescribed doses, Halt™ phosphatase inhibitor (Thermo-Fisher Scientific), and protease inhibitor cocktail (cOmplete™, Roche) for use in various biochemical assays. After centrifuging the homogenate at 15000 × g for 20 minutes, the supernatant was separated and stored for further biochemical examination. With the use of a colorimetric assay kit obtained from SolarBio (Beijing, China), the superoxide dismutase (SOD), catalase (CAT) and acetylcholinesterase (AChE) levels were determined. ELISA kits (colorimetric) from Sunlong Biotech Co., Ltd. (Hangzhou, China) were used to measure the levels of NFKB-65, CREB, COX-2, MBP, and NGF in accordance with the manufacturer's instructions.

Statistical analysis

The changes and comparisons between different parameters at the end of toxicity stage (week 5) and healing stage (week 7) were statistically analyzed using

One-way analysis of variance (ANOVA). One-way ANOVA was applied for intergroup comparisons whereas Tukey's post-hoc test applied for multiple comparisons. Alterations in the results were considered significant when the p value was ⩽ 0.05. Most of the statistical analyses were performed using Microsoft Excel and Social Science Statistics freely available at https://www.socscistatistics.com/

Results

Effects on Catalase Activity

Catalase (CAT) activity in the cerebral cortex was significantly decreased in the CPZ group following five weeks of CPZ administration (0.96 ± 0.09), compared with the control group (3.38 ± 0.65), indicating marked oxidative stress and impairment of endogenous antioxidant systems (Fig 1A). Neither discontinuation of CPZ nor the absence of treatment prevented this decline, confirming CPZ's capacity to disrupt cellular redox status. CFZ and PFZ treatments significantly restored CAT activity relative to CPZ, with PFZ demonstrating the most robust recovery, likely due to piperine-enhanced curcumin bioavailability. BFZ failed to improve CAT activity, confirming that active phytochemicals were required for measurable enzyme restoration. Following CPZ withdrawal, the CPZ group exhibited spontaneous CAT recovery (6.39 ± 0.84), while continued treatment further amplified CAT levels, with PFZ achieving the highest activity (7.92 ± 0.41), surpassing the control group (Fig 2A).

Effects on Superoxide Dismutase (SOD)

Five weeks of CPZ administration reduced SOD activity to 8.47 ± 0.56 compared with 15.59 ± 2.15 in controls, reflecting profound oxidative stress and mitochondrial impairment. BFZ treatment produced partial recovery, potentially attributable to intrinsic antioxidative properties of the ZRO-SNEDDS vehicle (Fig 1B). CFZ improved SOD levels moderately, whereas PFZ treatment resulted in the highest SOD values at week 7, surpassing
both CFZ and BFZ (Fig 2B). Enhanced curcumin bioavailability through piperine likely contributed to PFZ's superior antioxidative action. CPZ significantly suppressed MBP levels at week 5 (4.90 ± 1.11 vs. 9.36 ± 1.82), confirming demyelination (Fig 1D). At week 7, withdrawal of CPZ produced substantial spontaneous remyelination (16.79 ± 1.42). BFZ unexpectedly improved MBP to high levels, suggesting intrinsic myelin-supportive properties of ZRO-SNEDDS. CFZ increased MBP moderately, while PFZ achieved the highest MBP recovery (21.67 ± 2.64), exceeding control levels (Fig 2D), indicating enhanced remyelination linked to synergistic curcumin–piperine effects.

Effects on Acetylcholinesterase (AChE)

AChE levels were significantly reduced (Fig 1C) in the CPZ group at week 5 (24.47 ± 4.85 vs. 48.15 ± 7.63), consistent with CPZ-induced cholinergic dysfunction. PFZ preserved AChE activity most effectively ($p \leqslant 0.0001$). CFZ showed poorer protection, while BFZ provided modest support. After CPZ withdrawal, the CPZ group partially recovered. PFZ and BFZ led to markedly higher AChE levels by week 7 (Fig 2C), indicating enhanced cholinergic recovery mechanisms supported by improved curcumin bioavailability.

Effects on Myelin Basic Protein (MBP)

CPZ significantly suppressed MBP levels at week 5 (4.90 ± 1.11 vs. 9.36 ± 1.82), confirming demyelination (Fig 1D). At week 7, withdrawal of CPZ produced substantial spontaneous remyelination (16.79 ± 1.42). BFZ unexpectedly improved MBP to high levels, suggesting intrinsic myelin-supportive properties of ZRO-SNEDDS. CFZ increased MBP moderately, while PFZ achieved the highest MBP recovery (21.67 ± 2.64), exceeding control levels (Fig 2D), indicating enhanced remyelination linked to synergistic curcumin–piperine effects.

Effects on NF-κB-p65

CPZ dramatically elevated NF-κB-p65 activity (14.35 ± 2.54), confirming severe neuroinflammation at the end of Week 5 (Table 1). BFZ reduced this activation significantly, CFZ showed minimal suppression, while PFZ reduced NF-κB-p65 to near-control levels during CPZ exposure, demonstrating strong anti-inflammatory synergy. After CPZ removal, NF-κB-p65 levels declined spontaneously, but PFZ further reduced it below control values, highlighting its robust inflammatory suppression.

Effects on CREB Expression

CPZ reduced CREB to less than 30% of control values, demonstrating severe impairment of neuroplasticity pathways. BFZ offered mild protection, CFZ restored CREB to nearly 72% of control, and PFZ to nearly 65% (Table 2). After CPZ withdrawal, both CFZ and PFZ increased CREB to more than double control level, indicating strong neurotrophic and synaptic recovery. These results indicate that curcumin in its nanoformulation enhances CREB-mediated transcriptional activity far beyond normal physiological levels, potentially benefiting neuroregeneration and plasticity.

Effects on COX-2

Five weeks of CPZ provoked an 18-fold increase in COX-2 expression (1259.01 ± 84.36), confirming strong

inflammatory activation (Table 3). BFZ, CFZ, and PFZ significantly reduced COX-2, with PFZ showing maximal suppression. After CPZ withdrawal, COX-2 remained elevated in the CPZ-only group. PFZ achieved an 83.52% reduction relative to CPZ and a lower value than control, highlighting potent anti-inflammatory action

Effects on NGF Expression

NGF increased nearly fivefold following CPZ administration for Week 5 (128.13 ± 8.45 vs. 26.49 ± 3.22), indicating a compensatory neurotrophic response. BFZ moderately reduced NGF, CFZ and PFZ further lowered NGF expression, with PFZ showing the most pronounced suppression. After CPZ withdrawal, NGF remained elevated in the CPZ group, while PFZ reduced NGF below control levels, suggesting enhanced regulation of neuroinflammation (Table 4).

Treatment Group	NFKB-p65 (pg/mg protein)	
	Week 5	Week 7
CNT	5.09 ± 0.38	24.44 ± 3.12
CPZ	14.35 ± 2.54	29.27 ± 3.49
BFZ	8.29 ± 0.91	11.31 ± 1.35
CFZ	14.21 ± 2.52	15.55 ± 0.84
PFZ	6.25 ± 0.89	10.27 ± 1.08
p - value	CNT vs CPZ****; CNT vs CFZ****; CPZ vs PFZ**** CPZ vs BFZ**; CPZ vs PFZ** CNT vs BFZ* CPZ vs CFZ (ns); CNT vs PFZ (ns)	CNT vs CPZ*; CNT vs BFZ* CNT vs CFZ**; CNT vs PFZ** CPZ vs CFZ***, CPZ vs PFZ***;

Table 1: Effect of cuprizone and ZRO-based nanoformulations on NFKB-p65 level in cerebral corte. One-way ANOVA with Tukey's multiple comparison test was done to compare the variance between groups. *p ⩽ 0.05, ** p ⩽ 0.01, *** p ⩽ 0.001, **** p ⩽ 0.0001, ns = nonsignificant.

Treatment Group	CREB (pg/mg protein)	
	Week 5	Week 7
CNT	415.33 ± 34.68	227.41 ± 22.31
CPZ	124.23 ± 12.36	171.12 ± 19.85
BFZ	141.54 ± 11.28	132.10 ± 9.43
CFZ	297.80 ± 23.74	511.62 ± 41.36
PFZ	268.56 ± 18.64	476.80 ± 18.64
p - value	CPZ vs BFZ (ns); PFZ vs CFZ (ns) CNT vs CPZ***; CNT vs BFZ*** CNT vs PFZ*; CNT vs CFZ*; CPZ vs CFZ*; CPZ vs PFZ*	CNT vs CPZ*; CNT vs BFZ* CNT vs CFZ**; CNT vs PFZ** CPZ vs CFZ***, CPZ vs PFZ***; BFZ vs CFZ***, BFZ vs PFZ*** CPZ vs BFZ (ns); PFZ vs CFZ (ns)

Table 2: Effect of cuprizone and ZRO-based nanoformulations on CREB level in cerebral cortex. One-way ANOVA with Tukey's multiple comparison test was done to compare the variance between groups. *p ⩽ 0.05, ** p ⩽ 0.01, *** p ⩽ 0.001, **** p ⩽ 0.0001, ns = nonsignificant.

Treatment Group	NFKB-p65 (pg/mg protein)	
	Week 5	Week 7
CNT	68.18 ± 5.61	84.18 ± 7.26
CPZ	1259.01 ± 84.36	481.20 ± 37.63
BFZ	770.52 ± 52.14	163.11 ± 9.45
CFZ	522.23 ± 32.15	102.56 ± 7.64
PFZ	318.55 ± 15.48	79.28 ± 6.38
p - value	CNT vs CPZ****; CNT vs BFZ**** CNT vs CFZ***; CNT vs PFZ***; CPZ vs CFZ*** BFZ vs CFZ**; BFZ vs PFZ**; CPZ vs BFZ**; CFZ vs PFZ**	CPZ vs (CNT, PFZ, CFZ)**** CPZ vs BFZ*** BFZ vs (CNT, CFZ, PFZ)* CNT vs CFZ (ns), CNT vs PFZ (ns); CFZ vs PFZ (ns)

Table 3: Effect of cuprizone and ZRO-based nanoformulations on COX-2 level in cerebral cortex. One-way ANOVA with Tukey's multiple comparison test was done to compare the variance between groups. *p ⩽ 0.05, ** p ⩽ 0.01, *** p ⩽ 0.001, **** p ⩽ 0.0001, ns = nonsignificant.

Treatment Group	NFKB-p65 (pg/mg protein)	
	Week 5	Week 7
CNT	26.49 ± 3.22	51.35 ± 6.45
CPZ	128.13 ± 8.45	119.15 ± 8.62
BFZ	55.01 ± 3.87	76.91 ± 6.63
CFZ	17.46 ± 1.16	65.40 ± 4.34
PFZ	21.88 ± 1.53	45.76 ± 3.92
p - value	CPZ vs (CNT, CFZ, PFZ)*** BFZ vs (CNT, CPZ, CFZ, PFZ)** CNT vs CFZ, PFZ (ns) PFZ vs CFZ (ns)	**CPZ vs (CNT, CFZ, PFZ)** BFZ vs (CPZ, PFZ)* ; CNT vs BFZ* CNT vs CFZ & PFZ (ns)

Table 4: Effect of cuprizone and ZRO-based nanoformulations on NGF level in cerebral cortex. One-way ANOVA with Tukey's multiple comparison test was done to compare the variance between groups. *p ⩽ 0.05, ** p ⩽ 0.01, *** p ⩽ 0.001, **** p ⩽ 0.0001, ns = nonsignificant.

Discussion

The results demonstrate that CPZ exposure induces comprehensive oxidative, inflammatory, cholinergic, and myelin-related damage in the cerebral cortex, consistent with established CPZ neurotoxicity models [21,22]. The pronounced reduction in CAT and SOD aligns with CPZ-driven ROS generation, mitochondrial dysfunction, and depletion of endogenous antioxidative pathways [23,24]. Treatments containing curcumin, especially PFZ, effectively replenished antioxidant enzymes, reflecting curcumin's known Nrf2 activation and ROS-scavenging potential [25]. Piperine's ability to inhibit glucuronidation and enhance curcumin absorption [26,27] explains PFZ's superiority.

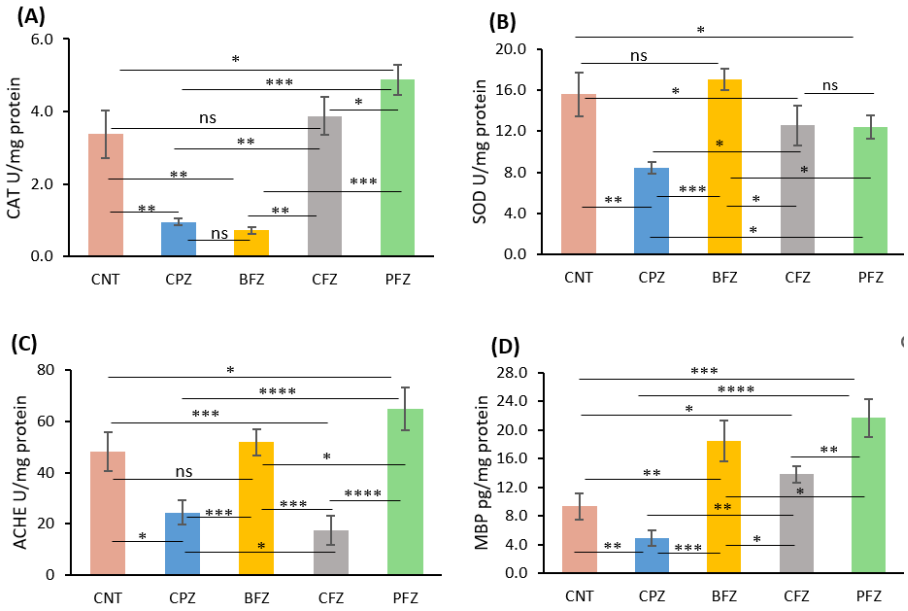

Figure 1: Effects of five-week prophylactic administration of cuprizone and ZRO-based nanoformulations on cortical levels of SOD, CAT, AChE, and MBP. *p ⩽ 0.05, ** p ⩽ 0.01, *** p ⩽ 0.001, **** p ⩽ 0.0001, ns = nonsignificant. Control (CNT); Cuprizone (CPZ); Blank oil formulation (BFZ); Curcumin nanoformulation (CFZ); and Curcumin with piperine nanoformulation (PFZ).

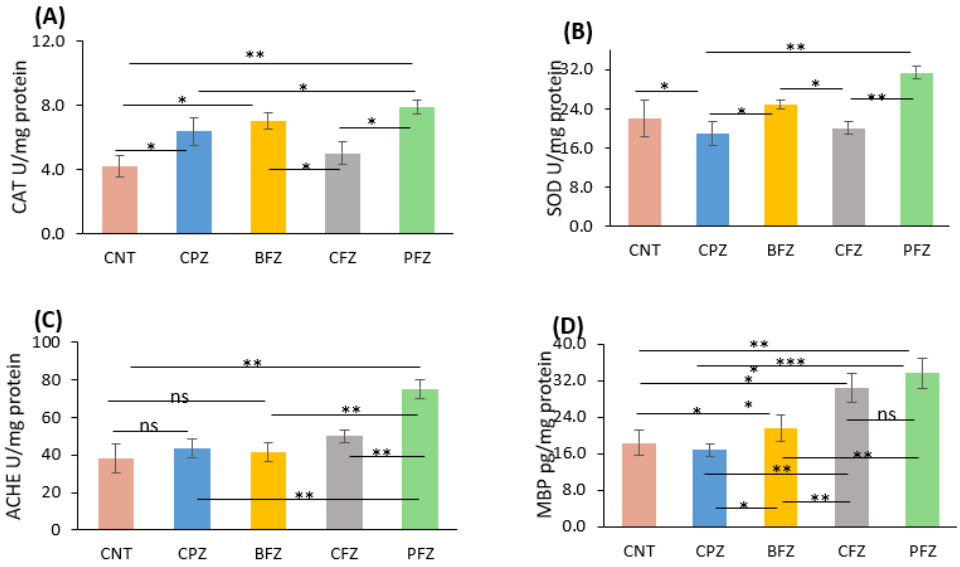

Figure 2: Effects of ZRO-based nanoformulations (therapeutic) administration for seven weeks on the levels of SOD, CAT, ACHE and MBP in cerebral cortex. After week 5, CPZ was withdrawn from rodent diet. *p ⩽ 0.05, ** p ⩽ 0.01, *** p ⩽ 0.001, **** p ⩽ 0.0001, ns = nonsignificant. Control (CNT); Cuprizone (CPZ); Blank oil formulation (BFZ); Curcumin nanoformulation (CFZ); and Curcumin with piperine nanoformulation (PFZ).

Neuroinflammation was strongly evident from elevated COX-2 and NF-κB-p65 levels, confirming activation of canonical inflammatory cascades [28,29]. PFZ's marked suppression of these markers reflects synergistic inhibition of NF-κB and downstream cytokines (TNF-α, IL-1β), consistent with established interactions between curcumin–piperine combinations and inflammatory pathways [30,31].

The sharp decline in CREB highlights CPZ's capacity to disrupt neuroplasticity [32,33]. Curcumin's ability to activate CREB and downstream neurotrophins [34] accounts for the substantial CREB upregulation in CFZ and PFZ groups. Enhanced CREB may promote long-term synaptic repair and cognitive resilience.

The observed demyelination and MBP reductions align with CPZ's known toxicity to oligodendrocytes [9,35]. PFZ achieved the greatest MBP restoration, likely due to combined antioxidative, anti-inflammatory, and neurotrophic effects facilitating remyelination. Surprisingly, BFZ also increased MBP, suggesting ZRO-SNEDDS may support lipid metabolism or myelin membrane stability [36].

NGF elevation following CPZ reflects neuronal injury signaling [37]. PFZ's ability to normalize NGF below control levels indicates fine regulation of neurotrophin signaling, with potential to prevent maladaptive overactivation [38,39]. Collectively, PFZ consistently outperformed CFZ and BFZ across all parameters. This reinforces the crucial role of optimized nanoformulations and bioenhancers in improving phytochemical therapeutic outcomes.

The present study demonstrates that cuprizone exposure induces notable alterations in oxidative, inflammatory, and neuroplasticity markers within the cerebral cortex, leading to neurotoxicity and demyelination. Prophylactic treatment with the curcumin–piperine nanoformulation (PFZ) showed potential in mitigating these effects by modulating antioxidant enzymes (CAT, SOD), reducing inflammatory markers (COX-2, NF-κB-p65), and improving indicators of myelin integrity (MBP) and synaptic function (CREB, AChE). Compared with blank oil (BFZ) and curcumin alone (CFZ), PFZ produced more consistent improvements across multiple parameters, likely due to the complementary pharmacological actions of curcumin and piperine. These findings suggest that PFZ may offer neuroprotective benefits against cuprizone-induced oxidative stress and inflammation. However, as this work is based on a preclinical mouse model, further studies are needed to confirm these results, elucidate underlying molecular mechanisms, and assess long-term safety and translational relevance.

We acknowledge the limitations in this study such as use of only male mice, lack of behavioral testing and mechanistic pathways inferred but not directly tested.

Ethical Approval

Animal studies were carried out in compliance with KFMRC's animal unit committee rules. The biomedical ethics research committee at King Abdulaziz University authorized the study protocol (approval No. ACUC-22-1-2 dated April 13, 2022), which complied with the guidelines established by the KFMRC's Animal Care and Use Committee. The study was authorized by Royal Decree No. M/59 dated August 24, 2010, and it conformed with the "System of Ethics of Research on Living Creatures" rules created by King Abdulaziz City for Science and Technology. The study was conducted at the King Fahad Medical Research Center in Jeddah, Saudi Arabia from June 1, 2022, until July 16, 2022.

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Conflict of Interest

Authors declared no conflict of interest.

Author Contribution

MZA conceived the idea and received the funding, AA analyzed the data, BSA designed the methodology and validated the results. MZA conducted *in vitro* experiments and wrote the manuscript.

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