Erythrina caffra Extract Restores Memory, Modulates Cholinergic Dysfunction, Neuroinflammation, and Attenuates Oxidative Stress in Cadmium-Induced Alzheimer's Disease-Like Pathology in Rats

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Abstract

Cadmium (Cd) is well known for its neurotoxic effects. Numerous studies have highlighted the link between its exposure and Alzheimer's disease (AD), considering that AD is a multifactorial disorder influenced by a complex interplay of various factors, including environmental factors. *Erythrina* species, including *Erythrina caffra*, are a rich source of bioactive substances with anti-inflammatory, antioxidant, and anticholinesterase activities. However, current studies on the preventive potential of *Erythrina caffra* against heavy metals linked to neurodegeneration are insufficient. This study explored the impact of cadmium on the cholinergic system, oxidative stress, neuroinflammation, and memory as key pathological features implicated in AD and the therapeutic potential of *Erythrina caffra* seeds ethanolic extract in attenuating cadmium-induced AD-like alterations in Wistar rats. Rats were exposed to cadmium chloride directly through intracerebroventricular injections. The treated groups received 2.5 mg/kg of *Erythrina caffra* extract and 20mg/kg of memantine via gavage. Memory performance, cholinergic dysfunction, oxidative stress, neuroinflammation, and neuronal integrity were assessed upon completion of the experiment. Results showed significant alterations in cholinergic function, evidenced by decreased levels of acetylcholine, decline in antioxidant enzyme activities, catalase, and superoxide dismutase, in addition to a significant decrease in Non-protein thiols level, an increase in the levels of IL-6 and TNF-α, and neuronal loss in the hippocampus. Both treatments restored memory, modulated cholinergic dysfunction and neuroinflammation, and prevented neuronal integrity and mitigated oxidative stress caused by cadmium. These findings suggest that *Erythrina caffra* may represent a promising therapeutic potential in mitigating key pathological features of AD.

Introduction

Cadmium (Cd) is a heavy metal that lacks a physiological function in the human body and is classified as a carcinogen [1]. It poses a health risk for both humans and animals [2, 3]. Prolonged exposure to Cd is associated with an elevated risk of different health issues, including kidney damage, osteoporosis, diabetes, cardiovascular disease, and several types of cancer [1, 2]. Furthermore, experimental studies have linked Cd exposure to cognitive deficits and Alzheimer's disease (AD) [1, 4].

Alzheimer's disease is a progressive neurodegenerative disorder that significantly affects cognitive function and behavior, with memory impairment being the most common initial symptom [2, 5]. The exact mechanisms underlying the pathogenesis of AD are still unclear. Numerous hypotheses have been proposed to explain its development, among these hypotheses are amyloid-beta (Aβ) accumulation, tau protein abnormalities, damage to cholinergic neurons, as well as oxidative stress and neuroinflammation [6–7]. Environmental factors, such as heavy metals, are considered important influencing factors in AD [2, 8]. However, their role in the pathogenesis of AD remains complex and not fully understood, despite their established neurotoxic effects. Cadmium is particularly concerning due to its harmful impact even at low levels of exposure. Given the widespread exposure of the general population to this metal, epidemiological studies have highlighted its association with the development of AD, underscoring the necessity of investigating this potent neurotoxin's contribution to the etiology of AD [8].

For its neurotoxicity, Cd enters the central nervous system (CNS) through two primary routes: oral ingestion, which can damage the blood-brain barrier (BBB), and inhalation, which bypasses the olfactory neuroepithelium and olfactory bulb [2]. Once in the CNS, Cd accumulates in neurons and exerts its neurotoxic effects through various mechanisms, one of which is oxidative stress. Cd triggers the generation of reactive oxygen species (ROS), leading to oxidative damage of proteins, lipids, and DNA in brain tissues. This oxidative stress is a key factor in Alzheimer's pathogenesis, as it promotes the aggregation of Aβ peptides [9]. Cadmium also interferes with tau protein, another critical pathological feature in AD, by accelerating its hyperphosphorylation. Hyperphosphorylated tau leads to the formation of neurofibrillary tangles, which further impair neuronal signaling and contribute to cognitive decline [10]. Beyond oxidative stress, Cd disrupts calcium homeostasis, which is essential for proper neuronal communication and synaptic plasticity. Cadmium mimics calcium ($Ca2^{+}$), entering neurons through $Ca2^{+}$ channels and disturbing intracellular $Ca2^{+}$ levels. This dysregulation contributes to mitochondrial dysfunction, reducing the energy supply for neurons and triggering apoptosis [11]. Additionally, cadmium is associated with chronic neuroinflammation, another significant contributor to AD. Cd activates microglia, eliciting the expression of proinflammatory cytokines, which exacerbate neuronal damage. Chronic neuroinflammation leads to a self-perpetuating cycle of oxidative stress and neuronal injury, accelerating the progression of AD [12].

Medicinal plants have garnered heightened interest due to their abundant bioactive compounds with significant antioxidants, anti-inflammatory, and neuroprotective properties. These natural compounds may counteract the neurotoxic effects of cadmium, thereby offering promising protection against Alzheimer's disease [13]. Their multifaceted mechanisms position them as valuable candidates for preventing or mitigating heavy metal-induced neurodegeneration. The Coast Coral tree, scientifically known as *Erythrina caffra Thunberg*, a member of the *Fabaceae* family, is well recognized for its historical use in traditional medicine. It's rich in bioactive flavonoids and alkaloids, exhibits potent antioxidants, anti-inflammatory, and neuroprotective properties [14]. However, its efficacy against heavy metal-induced neurotoxicity leading to cognitive decline and neurodegeneration remains insufficiently explored. In the present study, we aimed to evaluate whether intracerebroventricular (ICV) administration of cadmium chloride ($CdCl_2$) recapitulates key pathological features implicated in Alzheimer's disease, with a specific focus on cholinergic dysfunction, oxidative stress, and neuroinflammation. Additionally, we assessed the therapeutic potential of the ethanolic extract of *Erythrina caffra* (*E. caffra*) seeds in mitigating cadmium-induced AD-like alterations in Wistar rats.

Materials & Methods

Plant Collection and Extraction

The seeds of *E. caffra* were collected from the city of Kenitra. The species were identified, and samples were preserved in the Biology and Health laboratory herbarium at the Faculty of Science at Ibn Tofail University (Ref: EC-008/2021). The selected berries of *E. caffra* were dried overnight in an oven and then

ground. In a Soxhlet apparatus, the resulting fine powder was extracted with n-hexane for 6 hours at 50°C. After this first extraction, the suspensions were filtered, and the remaining n-hexane was removed using a rotary evaporator at 55°C. The defatted plant material was then positioned in an oven at 25°C overnight, followed by a second extraction with ethanol using the same Soxhlet apparatus to recover extracts [15, 16].

Animals and Housing

44 adult male Wistar rats, with an average weight of 309. 54 ± 4.36g and an age of 3 months were used in this study. To ensure their well-being, the rats were maintained in individual pens under standard conditions (Temperature of 24ºC and a 12-hour light/dark cycle). They were supplied with unrestricted availability of standard water and food. All experimental procedures utilized in the present study followed the guidelines outlined in the National Institute of Health's (NIH) Guide for the Care and Use of Laboratory Animals.

Stereotaxic Surgery

Prior to the surgery, animals were deeply anesthetized (7% chloral hydrate, i.p injection). Following anesthesia, the heads of rats were shaved, and they were securely fixed in a stereotaxic apparatus. The injection site was determined based on the rat brain atlas, ensuring it fell within the established normative range. Specifically, injections were targeted at coordinates averaging 0.8 mm in the anteroposterior direction, 1.5 mm in the medial-lateral direction, and 3.5 mm in the dorsoventral direction.

Experimental Design

The rats were randomly distributed into four groups (n = 11). The sham animals received an intracerebroventricular administration of sterile saline (5 µL/ventricle) using a Hamilton syringe. The disease group received a bilateral ICV injection of $CdCl_2$ (10 µg/kg; 5 µL/ventricle) (Sigma, St. Louis, MO, USA). The treated groups received similar $CdCl_2$ injections; one group was subsequently treated orally with the ethanolic extract of *E. caffra* seeds (via gavage) at a dose of 2.5 mg/kg, while the second treated group received oral memantine at a dose of 20 mg/kg, serving as the positive control group.

At the completion of the study, all rats underwent behavioral testing to evaluate memory performance. Thereafter, all rats were fasted overnight, weighed, anesthetized, sacrificed by decapitation, and the brain tissues were collected for histological and neurochemical analyses. These assessments focused on the hippocampus and included quantification of acetylcholine levels, determination of acetylcholinesterase activity, and evaluation of oxidative stress and neuroinflammatory markers (Fig. 1).

Memory Testing

Novel Object Recognition (NOR) Test

The NOR test was used to study short- and long-term memory (STM and LTM). The arena utilized consisted of a black cubic box (50x50x50 cm). Testing involved three phases and was conducted over three days. During day 1 (Habituation session), an individual animal was left to explore the device freely for 5 minutes. During day 2, each animal was replaced in the same testing apparatus with two identical objects (A/A). After a 2-hour interval (Testing session), the animals were tested again; however, during this phase, one of the familiar objects was randomly replaced with a novel object (A/B) to test STM. Each animal was allowed to freely explore the items for 5 minutes. The time spent exploring each item was recorded. After 24 hours, the animals were again placed in the arena for 5 minutes; the apparatus now included two items, the familiar item A and another unfamiliar item (C), to evaluate LTM [17].

Morris Water Maze (MWM) Test

To assess spatial learning and memory, a MWM test was employed. The apparatus comprised a water tank (40 cm in depth and 120 cm in diameter). The tank was partitioned into four points: North, South, West, and East. Within each quadrant, a visual signal was positioned on the outer surface of the labyrinth wall, serving as a reference point for the rats. An 11x14 cm platform was concealed beneath the water's surface, precisely 2cm deep, and positioned at the center of one of the four virtual quadrants. The platform remained stationary throughout the trials. During the training session, a total of five consecutive trials were conducted. In each trial, the rats were introduced into the tank, confronting the wall, starting from different positions, and given a maximum of 60 seconds to locate and reach the concealed platform. If a rat successfully reached the platform, it remained there for 10 seconds. If a rat failed to locate the platform within the allotted time, it was gently directed there [18]. The rat’s performance during the test period was recorded. The time it took the rat to locate the platform was measured.

Determination of Acetylcholine Level (ACh) and Acetylcholinesterase (AChE) Activity

Acetylcholine Level

The ACh level in the hippocampus was determined using the approach described by Augustinsson [19]. The hippocampus tissues were thermally incubated for precisely 5 minutes. Subsequently, the samples were treated with 0.5 mL of hydroxylamine hydrochloride (0.2M), 0.5 mL of sodium hydroxide (0.5M), and 0.5 mL of 50% hydrochloric acid. Following centrifugation at 10000 g for 10 minutes, 0.5 ml of ferric chloride (0.37M) was added to the supernatant. The optical density (OD) was assessed at 540 nm relative to a blank. The results were expressed as a percentage of control results.

Acetylcholinesterase Activity

Acetylcholinesterase is an essential enzyme involved in the breakdown of ACh. To assess AChE activity, the method developed by Ellman et al. [20] was employed. This technique relies on the hydrolysis of ACh by AChE, resulting in the production of thiocholine and acetic acid, which play an important role in memory. Thiocholine, once liberated, reacts with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), resulting in the formation of a yellow-colored product, 2-nitro-5-thiobenzoate. The intensity of the yellow color, measured at 412 nm, is proportional to the AChE activity [21]. On the day of analysis, the hippocampus tissues were extracted, weighed, and then pulverized and homogenized in a Tris/HCl buffer solution (50 mmol. L-1, pH 7) mixed with sucrose. The resultant homogenate was centrifuged, and the supernatants were carefully collected to test AChE activity using a spectrophotometer. The AChE results were expressed as a percentage of the control group.

Determination of Superoxide Dismutase (SOD) Activity

SOD is a vital antioxidant enzyme that is the first line of defense against oxidative stress. SOD activity in the hippocampus was measured using the methodology established by Beauchamp and Fridovich [22]. A 1 mL reaction mixture was generated by combining 0.94 mL of phosphate buffer (PB) (50 mM, pH 7.4) with 0.06 mL of supernatant. The PB containing riboflavin (2 µM), EDTA (0.1 mM), methionine (12 mM), nitroblue tetrazolium (NBT) (75 µM), and Triton X-100 (0.025%). The resulting mixture was exposed to yellow light for 10 minutes. Under the effect of oxygen and electron donors such as methionine, riboflavin's illumination produces superoxide anions, which inhibit NBT reduction. The control combination did not contain the enzyme source. The measurement of absorbance at 560 nm was utilized to determine the decrease of NBT to the blue-colored formazan, which indicates the presence of superoxide radicals. SOD activity was expressed as U/mL of supernatant.

Determination of Catalase (CAT) Activity

CAT activity was measured using the established method by Aebi [23]. 0.05 mL of supernatant was added to 1.95 mL of PB (0.05 M, pH 7.4) contained in a quartz cuvette. The reaction was initiated by the addition of 1 mL of H_2O_2 (0.05 M), and the decrease in absorbance was recorded over 2 minutes at 240 nm. This method enables the quantification of catalase activity by measuring the rate of hydrogen peroxide decomposition, which is directly proportional to CAT activity. The results were expressed as U/g of tissue.

Determination of Non-protein Thiols (NPSH) Level

NPSH is a key measure of thiol status, essential for antioxidant defense against oxidative stress, with glutathione (GSH) as its predominant form, comprising about 95% of total NPSH. GSH functions as a major antioxidant [24, 25]. Thus, assessing NPSH levels in the brain offers valuable insights into GSH status. In this study, NPSH levels were determined using Ellman’s method [29]. Brain supernatant was treated with trichloroacetic acid (TCA) (10%) and then centrifuged. The resultant supernatant was treated with 1 M potassium buffer (pH 7.4) and 1 mM DTNB. NPSH levels were measured at 412 nm using a spectrophotometer and expressed in µmol/g of tissue [25].

Assay for Lipid Peroxidation (LPO)

Malondialdehyde (MDA) is an important biomarker for LPO and oxidative stress, formed as a reactive aldehyde during the breakdown of polyunsaturated fatty acids. In this study, LPO was indirectly assessed by measuring MDA levels in homogenates from the hippocampus using the method of Draper and Hadley [26]. This assay is based on the reaction between MDA and thiobarbituric acid (TBA), producing a pink complex that absorbs light at 532 nm. The OD of the TBA-MDA complex, commonly referred to as TCA reactive substances (TBARS), is directly proportional to MAD level. To perform the assay, 1 mL of tissue homogenate was mixed with 1 mL of 10% TCA and centrifuged. Then, 1 mL of 0.67% TBA was added to the supernatant, and the mixture was heated at 100°C for 15 minutes to form the TBA-MDA complex. After cooling, TBARS levels were determined by measuring absorbance at 532 nm, with Malondialdehyde levels expressed as nmol per gram of tissue (nmol/g).

Nitrite/Nitrate Assay

To determine the concentration of nitrite in the hippocampus, the diazotization method was employed, which relies on the Griess reaction (GR). The Griess reaction is a commonly utilized indirect approach for assessing nitric oxide (NO) production [27]. In this method, samples were dispensed into tubes, and an equal volume of GR was added to each tube. The GR consisted of % sulfanilamide (1mL), N-1-naphthyl ethylenediamine dihydrochloride (0.1%) (1 mL), and 2.5% orthophosphoric acid. Following a 30-minute incubation at room temperature, the absorbance was measured at 540 nm. The quantification of nitrite levels in hippocampus tissues was expressed as µmol/g of tissue [28].

TNF-α and IL-6 Levels

TNF-α and IL-6 levels were measured by a sandwich ELISA. The procedure begins with the coating of ELISA plates using specific antibodies against TNF-α and IL-6 diluted in 1xPBS. The diluted antibody (100 µL) was added to each well of an enhanced protein-binding ELISA plate, followed by incubation overnight at 4°C. The capture antibody solution was washed with PBS. To block non-specific binding, 200 µL/well of Blocking Buffer was added, and the plate was incubated for 1 hour at room temperature. The plate was then rewashed with PBS. Subsequently, samples (50 µL/well) and standards were added and incubated for 1 hour to allow for the binding of target cytokines to the coated antibodies. Following another series of washes, 50 µL of biotinylated secondary antibody was added to facilitate detection, then washed and incubated for 2 hours. Subsequently, an avidin-horseradish Peroxidase (Av-HRP) conjugate or streptavidin-HRP was diluted to its optimal concentration in Blocking Buffer/Tween® and added (50µL/well), and incubated for 1 hour at room temperature. After washing the plates once more, 3,3’,5,5’tétraméthylbenzidine substrate solution (50µL) was added to allow color development. The reaction was terminated using H_2SO_4 solution, and the OD concentration for each well was measured using a microplate reader set to 450 nm. Results are expressed in picograms per milliliter (pg/mL) [29].

Histological Evaluation, Microscopic Examination, and Image Analysis

Nissl staining was employed to observe histopathological changes within the hippocampus. Brain tissue was fixed in neutral buffered formalin (10%), then embedded in paraffin and sectioned. Thin brain

sections were cut using a microtome (5µm). The slides were stained with 1% cresyl violet (Sigma-Aldrich) [42]. Under light microscopy, the stained sections of the hippocampus were examined, and images were captured with a digital camera. For quantitative analysis, sections were assessed over a standardized area of 3000 µm² using ImageJ software, a Java-based image processing tool from the NIH. Neurons were counted based on specific morphological criteria; cells with distinct round bodies and spherical nuclei were classified as normal neurons [31].

Statistical Analysis

The obtained data were presented as the mean ± standard error of the mean (SEM). Analyses were performed using statistical software packages; the Shapiro-Wilk test was used to assess the normality of the data distributions. A One-Way or Two-Way ANOVA was used to evaluate whether there were statistically significant differences between group means. Post-hoc analyses were conducted to pinpoint specific group differences. Statistical significance was set at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Results

Memory Testing

In the NOR test (Fig. 2), animals that received ICV-$CdCl_2$ injections showed a significant decline in the recognition index for STM ($F_{3, 28} = 12.40$, $p < 0.0001$) and LTM ($F_{3, 28} = 27.82$, $p < 0.0001$) compared to the sham group. However, a significant rise in this index was observed in the treated group with the extract compared to the $CdCl_2$ untreated group ($p = 0.0001$). The memantine-treated group showed similar results.

In the MWM test (Fig. 3), the ethanolic extract of *E. caffra* significantly improved the spatial learning and memory of rats exposed to ICV-$CdCl_2$ injections. The findings show that $CdCl_2$ significantly increased the daily average latency to escape the maze compared to the sham group ($F_{3, 112} = 16.41$, $p < 0.0001$). However, a significant decline was observed following treatment with the extract ($p < 0.01$) and memantine ($p < 0.001$) compared to the untreated group (Fig. 3a). During the probe trial (Fig. 3b), the extract of *E.caffra* significantly increased the time spent in the correct quadrant compared to the $CdCl_2$ untreated group ($F_{3, 28} = 7.802$, $p = 0.0006$). A comparable result was observed in the memantine-treated group, although the increase was not statistically significant ($p > 0.05$).

Acetylcholine Level and Acetylcholinesterase Activity

As shown in Fig. 4a, $CdCl_2$ significantly decreased ACh levels in the hippocampus compared to the sham group ($F_{3, 20} = 14.39$, $p < 0.0001$). In contrast, an enhancement in hippocampal ACh levels was observed in *E. caffra* ($p = 0.0001$) and memantine-treated ($p = 0.0013$) groups compared to the $CdCl_2$ untreated group. Additionally, $CdCl_2$ increased AChE activity ($F_{3, 20} = 66.89$, $p < 0.0001$) (Fig. 4b); however, both treatments significantly reduced this enzymatic activity relative to the $CdCl_2$ group.

Brain Oxidative Stress Markers

As shown in Fig. 5, there is a significant reduction in SOD activity ($F_{3,20}$ = 8.993, p = 0.0006), CAT activity ($F_{3,20}$ = 8.608, p = 0.0007), and NPSH levels ($F_{3,20}$ = 13.24, p < 0.0001) following exposure to $CdCl_2$ compared to the sham group. However, both *E. caffra* and memantine significantly elevated SOD activity (p < 0.01) compared to the $CdCl_2$ untreated group (Fig. 5a). No significant changes in CAT activity were observed following either treatment (p > 0.05) (Fig. 5b). While there was a significant rise in NPSH levels in *E.caffra* (p < 0.001) and memantine (p < 0.01) treated groups relative to the untreated group (Fig. 5c).

The ICV-$CdCl_2$ injections significantly increased MDA ($F_{3, 20}$ = 8.380, p = 0.0008) and NO ($F_{3, 20}$ = 6.855, p = 0.0023) levels compared to the sham group. In contrast, both treatments decreased MDA levels in the hippocampus. Yet, this decrease did not reach statistical significance (p > 0.05) (Fig. 6a). Additionally, a significant elevation in NO levels was observed in rats treated with *E.caffra* (p < 0.05) and memantine (p < 0.01) compared to the $CdCl_2$ untreated rats (Fig. 6b).

Pro-Inflammatory Cytokines Levels

The ICV-$CdCl_2$ administration significantly increased the levels of TNF-α ($F_{3, 20}$ = 10.75, p = 0.0002) and IL-6 ($F_{3, 20}$ = 6.271, p = 0.0036) in the hippocampus compared to the sham group. Meanwhile, both treatments decreased the elevated levels of TNFα and IL-6 compared to the untreated $CdCl_2$ group (Fig. 7).

Histological Analysis

Results from Nissl-stained hippocampal sections in the sham group reveal large pyramidal neurons with distinct cell bodies, round nuclei, and border cytoplasm. Their processes are visible in the molecular layer. The pyramidal cells are densely packed, forming a continuous and organized layer. In the dentate gyrus (DG), granular neurons appear small, tightly arranged in many rows. No signs of nuclear pyknosis, cytoplasmic shrinkage, or other pathological alterations are observed (Fig. 8a). In contrast, histological examination of these sub-regions in the $CdCl_2$ group reveals a high prevalence of abnormal neuronal morphology. Pyramidal and granular neurons exhibit deformed, pyknotic cell bodies with poorly defined nuclei and cytoplasmic shrinkage, indicating cell death. A marked reduction in the density of pyramidal and granular neurons is also observed, with fewer normal cells present and disorganized neurons. However, treatment with the extract of *E.caffra* and memantine reveals normal neuronal morphology similar to that of the sham group. Neurons in these regions displayed well-defined pyramidal and granule cell bodies arranged in continuous and organized layers.

Histological analysis revealed significant decrease in the number of intact neuronal cells within the hippocampal subregions CA1 ($F_{3, 8}$ = 8.291, p = 0.0077), CA3 ($F_{3, 8}$ = 11.45, p = 0.0029) and DG ($F_{3, 8}$ = 5.088, p = 0.0293) in $CdCl_2$ group compared to the sham group (Fig. 8b). However, both treatments

raised the number of cells compared to the $CdCl_2$ untreated group, whereas this increase was not statistically significant ($p > 0.05$). Except in the CA3, the *E. caffra* significantly increased the number of cells ($p < 0.05$) compared to the $CdCl_2$ untreated group.

Discussion

E. caffra is one of the most used species of the genus *Erythrina* due to its biological activities and therapeutic potential [14, 32]. Previous studies have reported the antioxidant properties, anti-inflammatory effects, and the acetylcholinesterase inhibitory potential of alkaloids derived from *E. caffra*, which modulate key molecular pathways involved in AD and contribute to memory enhancement [33, 34].

The cholinergic system plays a central role in memory processes, and in AD, cholinergic transmission is impaired, leading to cognitive decline [33]. The results of the current study showed that administering $CdCl_2$ via the ICV route resulted in significant changes in the cognitive abilities of rats, causing alterations in memory. These changes were accompanied by increased oxidative stress, neuroinflammation, and neuronal loss in the hippocampus, an essential region for memory and one of the brain areas affected by Alzheimer's [35]. Whereas daily administration of the ethanolic extract of *E. caffra* seeds significantly impacted cognitive changes, biochemical alterations, and histological abnormalities attributable to cadmium exposure. These findings indicate that cadmium induced AD-like pathological changes and highlight the potential therapeutic effects of the *E. caffra* seeds extract in attenuating these pathological manifestations of AD.

The neurotoxic effects of Cd are well documented, as it is linked to cognitive decline and the onset of neurodegenerative disorders such as AD [8]. The findings from the NOR and MWM tests indicate that $CdCl_2$ significantly impairs the memory of experimental rats. Previous studies have demonstrated that Cd is associated with memory deficits in various animal models [36, 37]. The mechanisms underlying memory deficits following Cd exposure include dysregulation of the cholinergic system, oxidative stress, and neuroinflammation.

The cholinergic system dysfunction in the ICV-$CdCl_2$ animals is consistent with research indicating that exposure to Cd alters AChE activity and disrupts ACh levels. These changes directly impact synaptic transmission and cognitive functions, resulting in cognitive deficits observed in AD-like conditions [8, 38]. Cd is known to disrupt the Ca^{2+} balance in neurons. It interferes with calcium signaling pathways, which are crucial for neurotransmitter release and neuronal excitability. This disruption leads to impairment in the normal influx of Ca^{2+} necessary for the exocytosis of acetylcholine-containing vesicles [39]. More specifically, Cd enters neurons via dihydropyridine-sensitive (L-type) voltage-gated Ca^{2+} channels, where it competes with Ca^{2+} within the channel pore and blocks Ca^{2+} influx at physiological voltages. Consequently, this alteration in the normal influx of Ca^{2+} impairs ACh release [4]. Cd also directly affects cholinergic receptors, inducing alterations in cholinergic muscarinic receptors and AChE variants. Specifically, Cd disrupts M1 and M3 muscarinic receptors, leading to the overexpression of AChE-S and the downregulation of AChE-R. This dysregulation contributes to the degeneration of cholinergic neurons

and impairs cholinergic transmission [4, 39]. From the above, $CdCl_2$ negatively impacts the cholinergic system, leading to elevated AChE activity. AChE is known to interact with amyloid precursor protein and presenilin-1, which leads to the production of Aβ. This interaction contributes to the accumulation of Aβ plaques, an essential hallmark of AD pathology [40].

The enhancement in memory in the treated group with *E. caffra* extract was associated with increased hippocampal ACh levels, alongside reduced AChE activity in this region. In parallel with these neurochemical and cognitive improvements, histological examination further confirmed the neuroprotective effect of the *E. caffra*. The hippocampal subfields exhibited preserved neuronal morphology, characterized by intact pyramidal and granule cell layers with minimal signs of degeneration. These findings strongly suggest that the extract not only modulates cholinergic transmission but also protects hippocampal neurons from $CdCl_2$-induced neurodegeneration. These results are consistent with prior research, particularly the cholinergic hypothesis of AD, which posits that memory impairment in AD is associated with dysfunction of the cholinergic system due to increased AChE activity [41, 42] and decreased ACh level and availability in the synaptic cleft. ACh is a crucial neurotransmitter that plays an essential role in memory function [43]. These observations suggest that therapeutic interventions targeting AChE activity in the brain may be vital for mitigating cognitive decline associated with AD. Previous research on *Erythrina* alkaloids has indicated their potential as AChE inhibitors [33, 34]. Specifically, alkaloids from the genus *Erythrina*, including erythraline, erythrinine, and cristanine A, have shown promising inhibitory potential against human acetylcholinesterase, as confirmed through in vitro testing [44]. These alkaloids bind strongly within the active site of the enzyme, forming salt bridges with key residues like Trp86 and Tyr337, and engaging in hydrophobic interactions that stabilize their binding. This interaction prevents the enzyme from hydrolyzing ACh, thereby reducing its activity and alleviating symptoms of neurodegenerative diseases [44]. Furthermore, another study conducted by Gelfuso et al. [45] on the effects of *Erythrina*-derived alkaloids on memory in a rat model of epilepsy aligns with these findings. This study demonstrated that 11α-hydroxy-erythravine and erythravine alkaloids isolated from *Erythrina mulungu* exert neuroprotective effects on the hippocampus primarily by modulating cholinergic transmission through their antagonistic action on nicotinic ACh receptors (nAChRs). By inhibiting ACh-activated currents in cells expressing nAChRs, these alkaloids help regulate neuronal excitability and prevent neuronal damage. This neuroprotection preserves the structural integrity of hippocampal neurons, reducing cell death. Additionally, because nAChRs are critical for cognitive functions, including learning and memory, their modulation by the alkaloids results in enhanced cognitive performance. The preservation of hippocampal neurons and the stabilization of cholinergic signaling collectively contribute to improved memory and learning capabilities [45].

The imbalance in oxidative stress markers in the hippocampus of $CdCl_2$ animals was reduced in treated animals with *E. caffra* and memantine, suggesting that increased oxidative stress may play a crucial role in AD pathogenesis and that the extract may have beneficial effects on brain oxidative stress. Gella and Durany [46] demonstrated that the imbalance between ROS production and antioxidant defense, which leads to oxidative stress, plays a major role in neurodegeneration. Previous studies are consistent with

our findings regarding the beneficial effects of *Erythrina caffra* on brain oxidative stress. These studies demonstrated that *Erythrina caffra* exhibits strong antioxidant activities [11, 47]. Notably, the essential oil extracted from *E. caffra* has been shown to decrease oxidative stress markers while enhancing antioxidant enzyme levels, including CAT and SOD [47]. Furthermore, various species within the genus *Erythrina*, including *Erythrina indica*, *Erythrina senegalensis*, and *Erythrina × neillii*, have demonstrated antioxidant activities, where extracts from these plants restored the activities of CAT, SOD, and GSH [32].

Neuroinflammation has been reported to contribute to AD [48]. In the present study, the direct injection of Cd in the brain caused an elevation in TNF-α and IL-6 levels, which are among the primary proinflammatory cytokines activated in the CNS during neuroinflammatory responses. While the extract of *E. caffra* modulates the alteration in the level of these proinflammatory cytokines, indicating that the extract exerts immunomodulatory activity and offers neuroprotection against inflammatory processes associated with neurodegeneration caused by cadmium. Previous studies have shown that Cd can induce neuroinflammation by mechanisms involving BBB leakage, microglia activation, and infiltration of immune cells into the brain [48, 49]. Whereas *Erythrina* species, including *E. caffra*, modulate neuroinflammation primarily through their bioactive substances, which exhibit potent antioxidant and anti-inflammatory properties [32]. These compounds inhibit key inflammatory pathways by suppressing proinflammatory cytokines, as well as downregulating the NF-κB signaling cascade. Additionally, certain alkaloids in *Erythrina* act on nAChRs, engaging the cholinergic anti-inflammatory pathway, which further reduces microglial activation and neuroinflammation. Together, these mechanisms contribute to the neuroprotective effects of *Erythrina* against inflammation-induced neurodegeneration [32, 50].

Conclusion

In conclusion, our research highlights the neurotoxic potential of cadmium in inducing Alzheimer-like pathology. The present findings demonstrate that intracerebroventricular administration of $CdCl_2$ in rats replicates pathological features of AD, including increased oxidative stress, disruption of cholinergic transmission, neuroinflammation, hippocampal degeneration, and memory impairments. On the other hand, the ethanolic extract of *Erythrina caffra* seeds demonstrated neuroprotective effects. The daily administration of this extract over two months reduced oxidative stress by enhancing antioxidant defenses, modulating cholinergic neurotransmission and neuroinflammation, and preserving the structural integrity of hippocampal neurons. These combined effects contributed to the observed improvement in memory. These findings suggest that the ethanolic extract of *Erythrina caffra* seeds may represent a promising therapeutic potential in mitigating key pathological features of Alzheimer's disease.

Abbreviations

Aβ	Amyloid-beta
ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer’s disease
BBB	Blood-brain barrier
Cd	Cadmium
$CdCl_2$	Cadmium chloride
CAT	Catalase
CNS	Central nervous system
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
E. caffra	*Erythrina caffra*
GSH	Glutathione
ICV	Intracerebroventricular
IL-6	Interleukin-6
LTM	Long-term memory
MDA	Malondialdehyde
MWM	Morris Water Maze
NBT	Nitroblue tetrazolium
NIH	National Institute of Health
NO	Nitric oxide
NOR	Novel object recognition
NPSH	Non-protein Thiols
OD	Optical density
ROS	Reactive oxygen species
SOD	Superoxide dismutase
STM	Short-term memory
TNF-α	Tumor necrosis factor-alpha

Declarations

Competing Interests

The authors declare no competing interests.

Ethics Approval

The experimental procedures involving animals were conducted in accordance with the Animal Ethics Committee (Local Institutional Research Committee). Efforts were made to minimize animal suffering.

Author Contribution

Soumia Ed-Day, Fatima-Zahra Azzaoui, and Samira Boulbaroud: Conceptualisation & development of the study design. Radia ElGui, Latifa Didou, Laila Ibouzine-dine, Chaimae El Kourchi,Azzouz Haddan, and Hicham Harhar: Methodology, data collection, and analysis. Fatima Ezzahra Kacimi: Methodology, review & editing. All authors read and approved the final manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

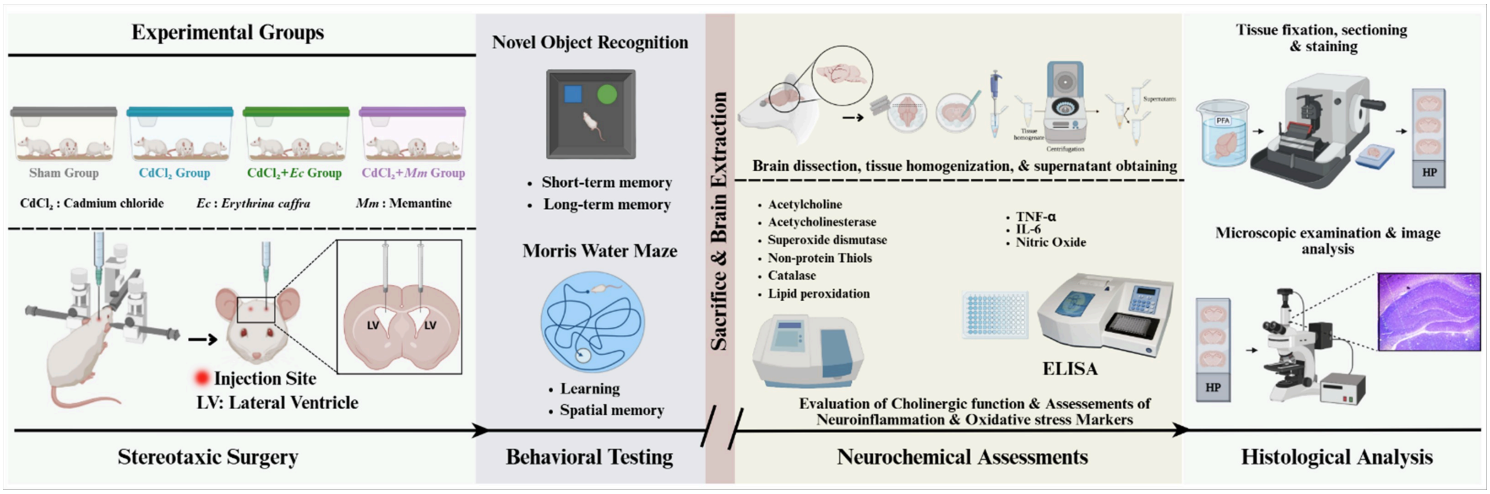

Figure 1

Experimental design of the study

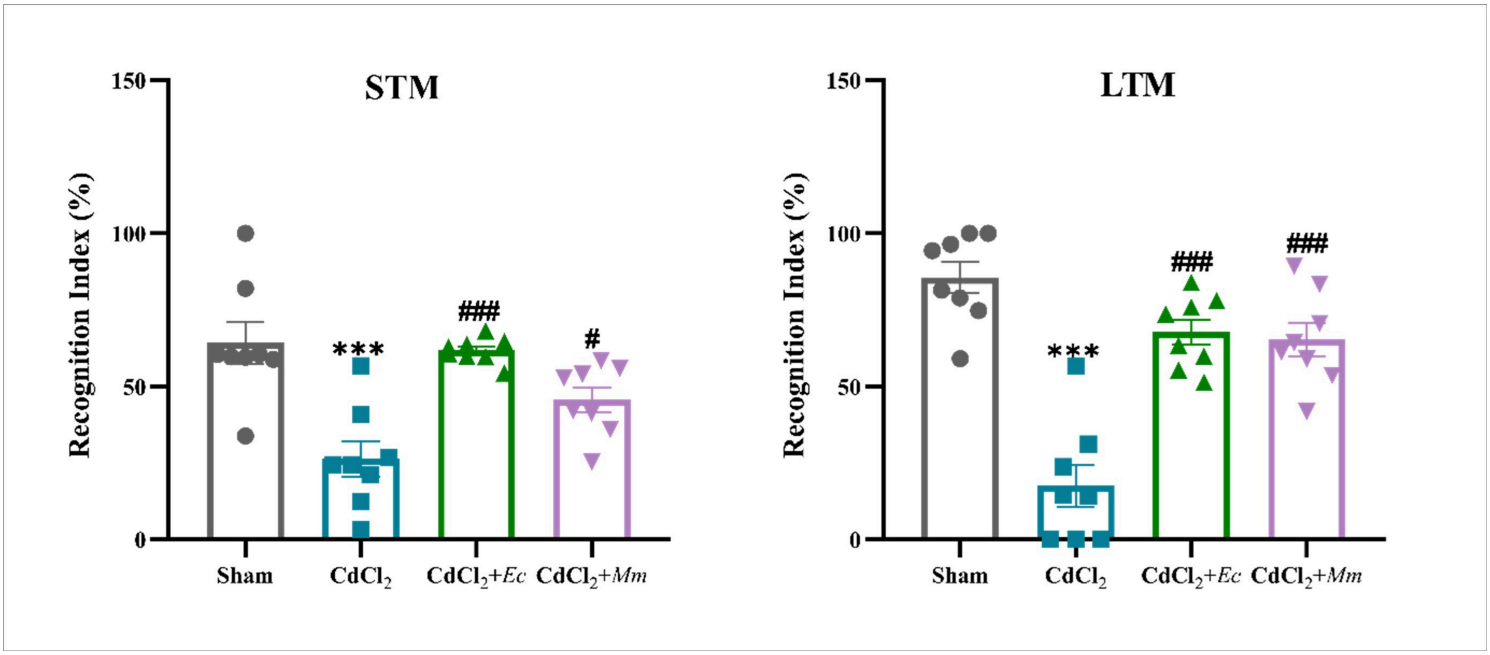

Figure 2

Effects of *Erythrina caffra* seeds ethanolic extract on memory evaluated in the novel object recognition test (n = 8).

*Data are presented as Mean ±SEM. The level of significance is 0.05 (One-way ANOVA test, followed by Tukey's multiple comparisons test). ***p < 0.001 compared with sham group; #p < 0.05, ##p < 0.01 and ###p < 0.001 compared with $CdCl_2$ group.*

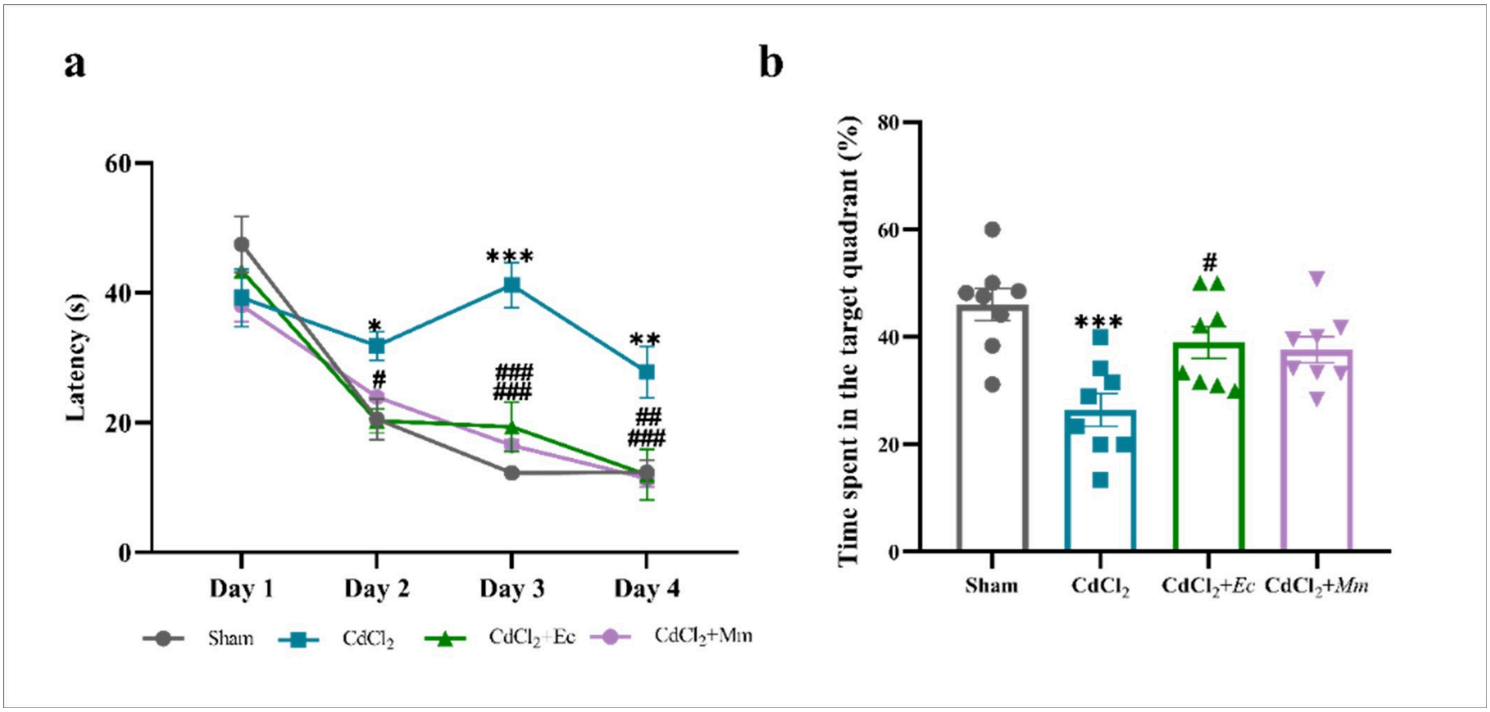

Figure 3

Effects of *Erythrina caffra* seeds ethanolic extract on memory performance of rats evaluated in the Morris Water Maze test (n = 8).

*Data are presented as Mean ±SEM. The level of significance is 0.05 (One-way and two-way ANOVA tests, followed by Tukey's multiple comparisons test). *p < 0.05, **p < 0.01 and ***p < 0.001 compared with sham group; #p < 0.05, ##p < 0.01 and ###p < 0.001 compared with $CdCl_2$ group*

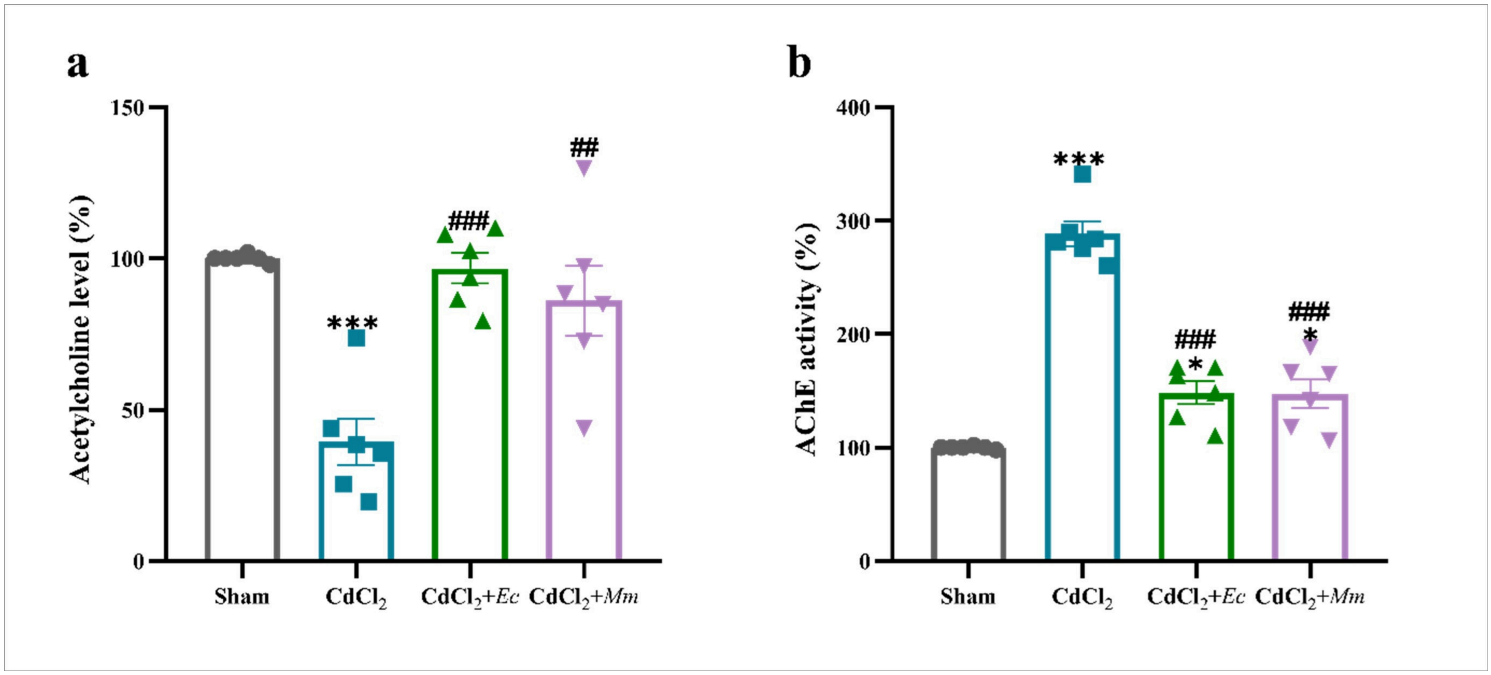

Figure 4

Effect of *Erythrina caffra* seeds ethanolic extract on the cholinergic system. **a.** Acetylcholine levels and **b.** Acetylcholinesterase activity in the hippocampus (n = 6).

*Data are presented as Mean ±SEM. The level of significance is 0.05 (One-way ANOVA test, followed by Tukey's multiple comparisons test). *p < 0.05 and ***p < 0.001 compared with sham group; ##p < 0.01 and ###p < 0.001 compared with $CdCl_2$ group*

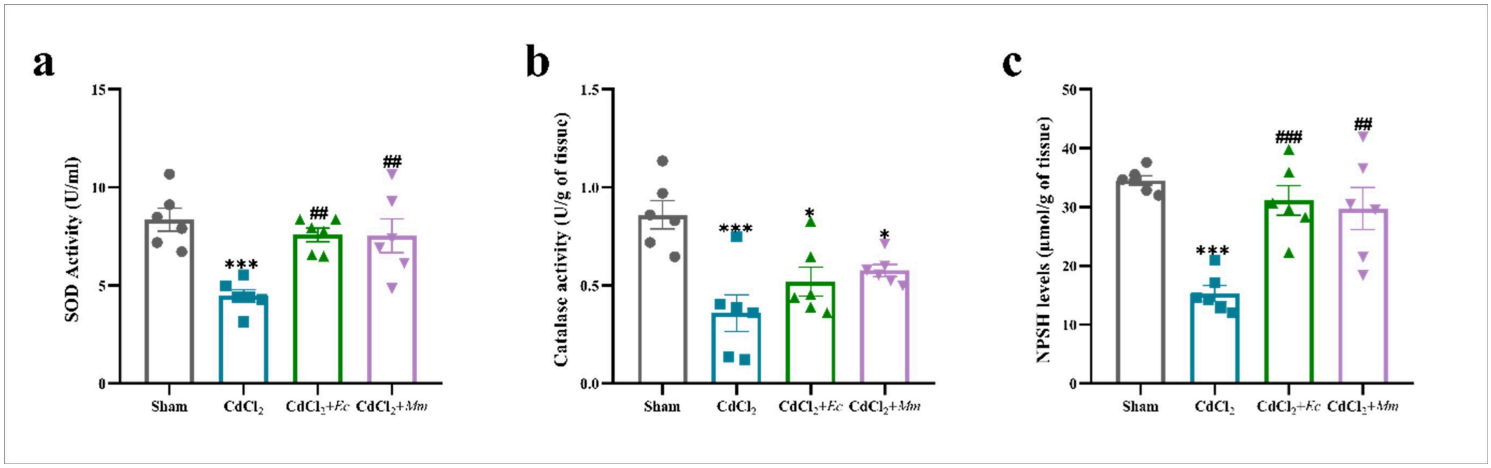

Figure 5

Effect of *Erythrina caffra* seeds ethanolic extract on antioxidant markers in the hippocampus. **a.** Superoxide dismutase activity, **b.** Catalase activity, and **c.** Non-Protein Thiol levels (n = 6).

*Data are presented as Mean ±SEM. The level of significance is 0.05 (One-way ANOVA test, followed by Tukey's multiple comparisons test). *p < 0.05 and ***p < 0.001 compared with sham group; ##p < 0.01 and ###p < 0.001 compared with the $CdCl_2$ group*

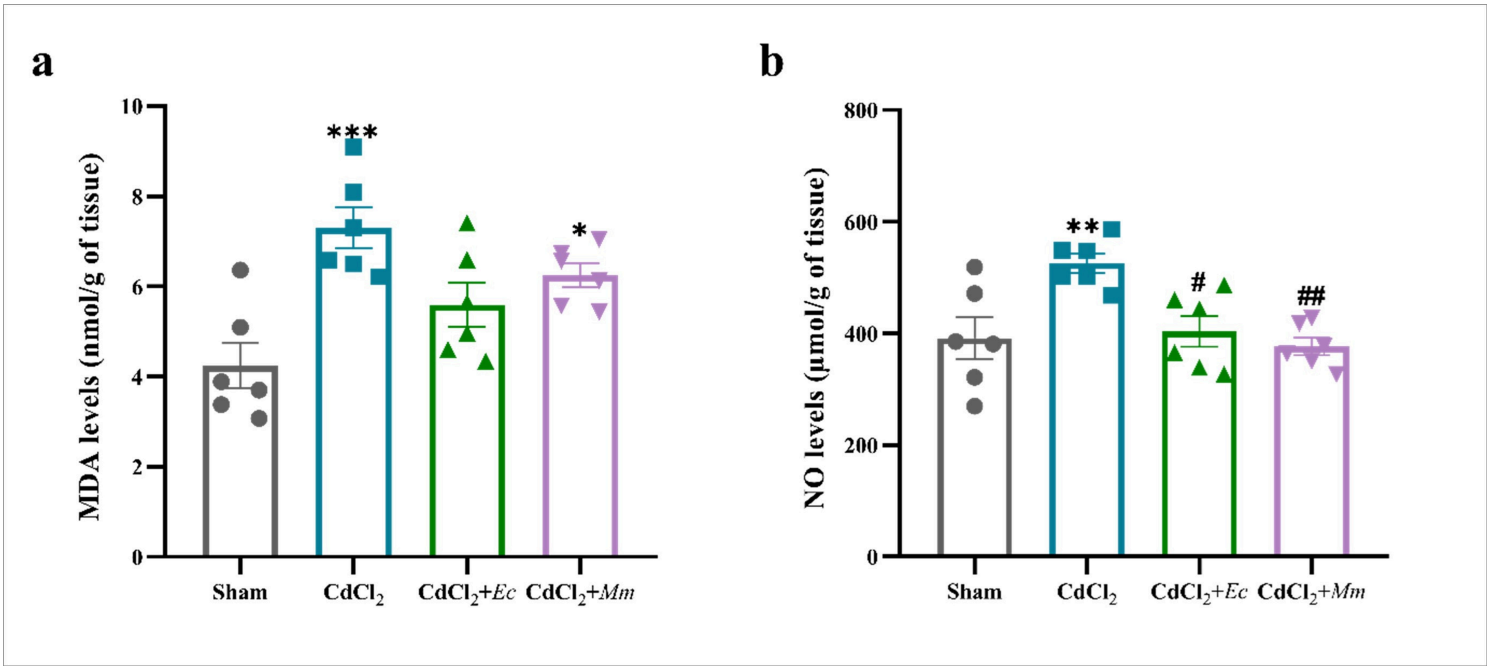

Figure 6

Effect of *Erythrina caffra* seeds ethanolic extract on **a.** Malondialdehyde levels, and **b.** nitric oxide levels in the hippocampus (n = 6).

*Data are presented as Mean ±SEM. The level of significance is 0.05 (One-way ANOVA test, followed by Tukey's multiple comparisons test). *p < 0.05, **p < 0.01, and ***p < 0.001 compared with sham group; #p < 0.05 and ##p < 0.01 compared with $CdCl_2$ group*

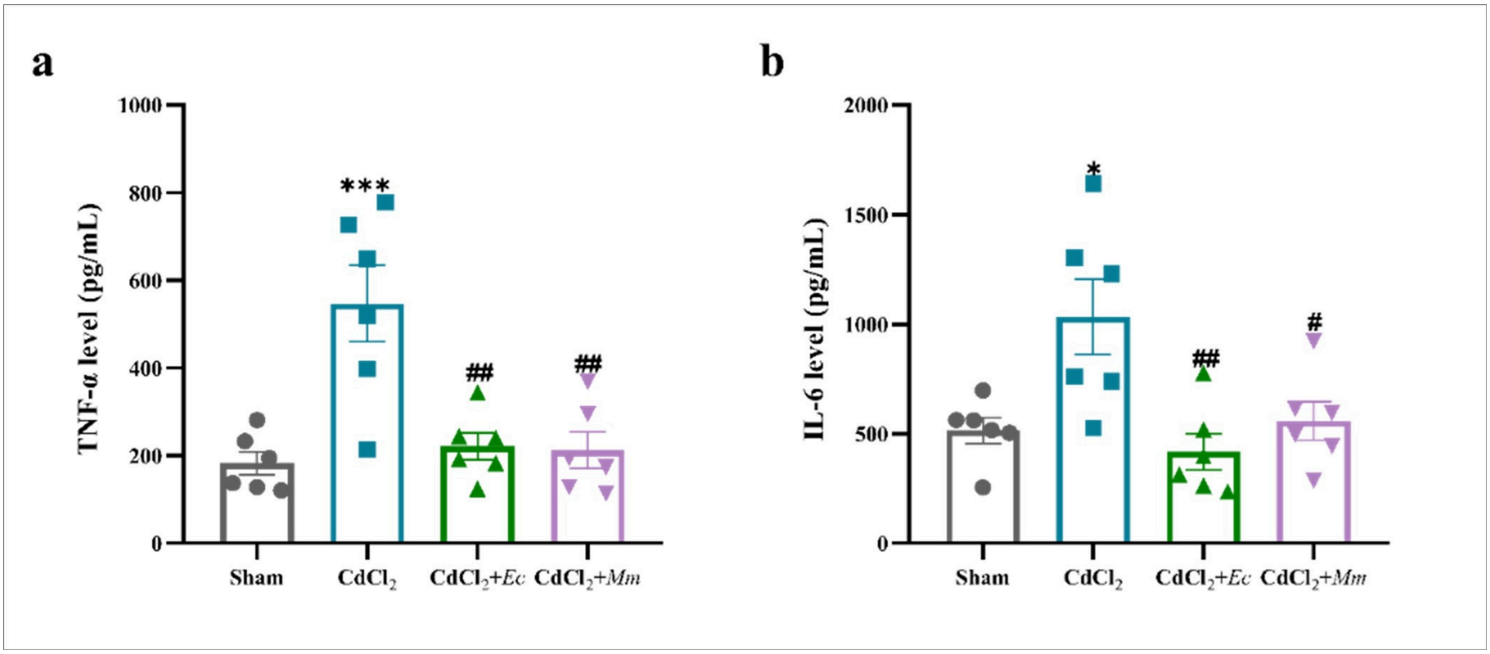

Figure 7

Effect of *Erythrina caffra* seeds ethanolic extract on pro-inflammatory cytokines in the hippocampus: **a.**Tumor Necrosis Factor-alpha (TNF-α) levels and **b.** Interleukin 6 (IL-6) levels (n=6).

*Data are presented as Mean ±SEM. The level of significance is 0.05 (One-way ANOVA test, followed by Tukey's multiple comparisons test). *p < 0.05, **p < 0.01, and ***p < 0.001 compared with sham group; #p < 0.05 and ##p < 0.01 compared with $CdCl_2$ group*

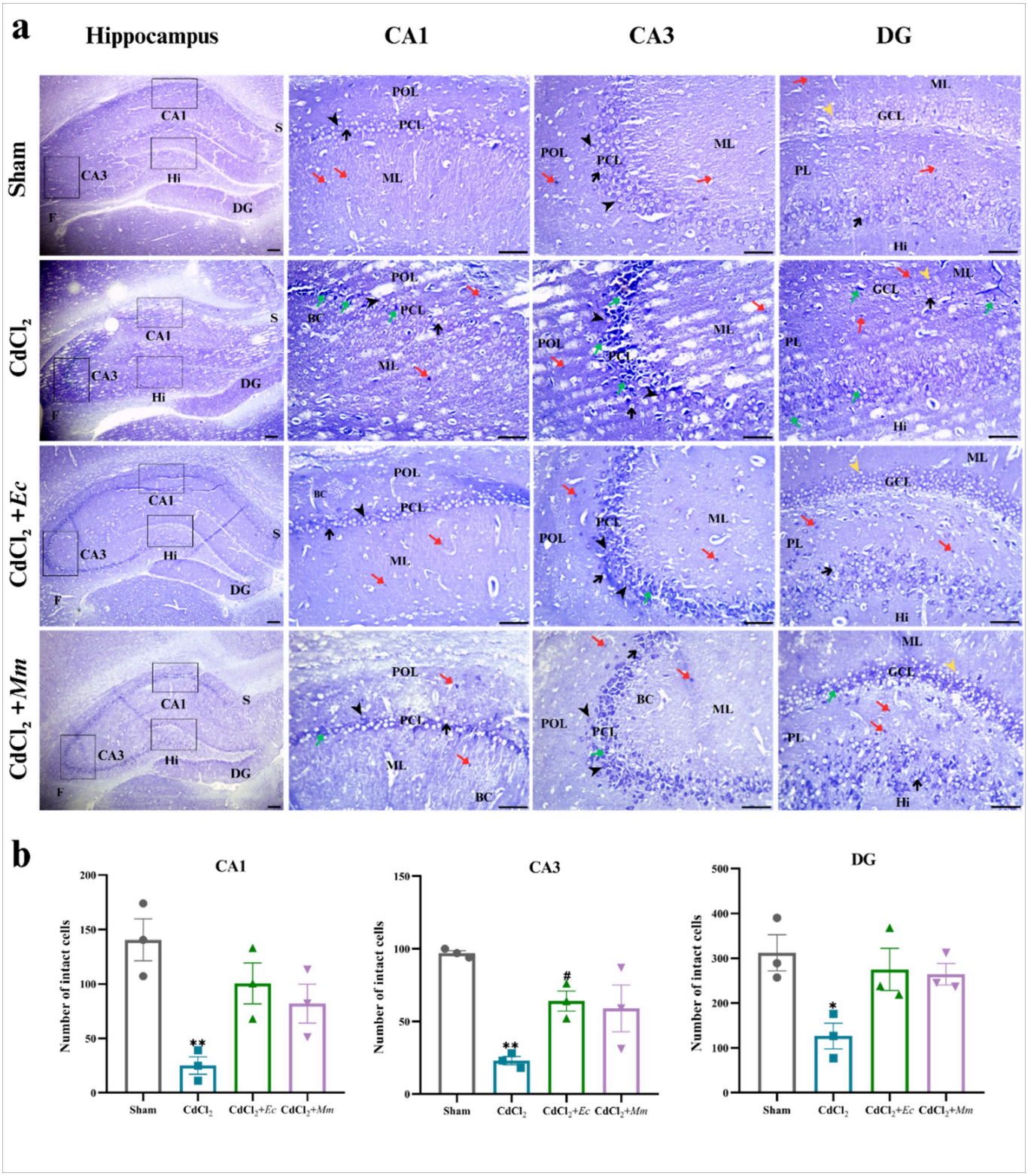

Figure 8

Effect of *Erythrina caffra* seeds ethanolic extract on hippocampal neurons damage induced by ICV-$CdCl_2$ injections. **a.** Nissl-stained microphotographs of coronal sections of rats' brains showing the hippocampus (x4 magnification) and its different parts: Cornu Ammonis (CA) with its two regions: CA1 and CA3, dentate gyrus (DG), and hilus (Hi). The fimbria (F) and subiculum (S) can be observed. Microphotographs of the squared area of the CA1 region contain three well-defined layers: the

polymorphic layer (POL), the pyramidal cell layer (PCL), and the molecular layer (ML). Microphotographs of the squared area of CA3 with its three distinct layers: POL, PCL, and ML. Microphotographs of the squared area of the DG showing three layers: ML, pleomorphic layers (PL), and granule cell layer (GCL); the Hi can be observed. Black arrowheads indicate cell bodies of the pyramidal neurons; short black arrows indicate prominent nucleoli; red arrows indicate glial cells; green arrows indicate degenerated neurons; yellow arrowheads indicate granule cell bodies of the granular neurons; and BC indicates blood capillaries (x20 magnification; scale bar = 100 μm). **b.** Number of intact cells in the hippocampal subregions CA1, CA3, and DG.

*The results are presented as Mean ±SEM. The significance level is 0.05 (One-way ANOVA test, followed by Tukey's multiple comparisons test). *p < 0.05 and **p < 0.01 compared with sham group; #p < 0.01 compared with $CdCl_2$ groups*