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Research Article

Neuroprotective (Antioxidant, Antiamyloidogenic, and Antiexcitatory) Effects of Trévo™ against Cadmium Chloride Neurotoxicity in Adult Male Wistar Rats

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Abstract

Cadmium (Cd) is a heavy metal that is deleterious to brain development as it increases brain aging. Trévo is a multi-herbal supplement that provides various health benefits, including boosting the immune system and detoxification. In this study, we investigated the neuroprotective effects of Trévo against the neurotoxic effects of cadmium chloride ($CdCl_2$). Thirty male Wistar rats were equally divided into three groups: Group I (normal control), Group II (administered $CdCl_2$), and Group III (administered Trévo and $CdCl_2$), and were used in the experiments. Animals were pretreated with 2 mL/kg of Trévo for five days and injected with Cd intraperitoneally 3 h later. Cd significantly increased the production of malondialdehyde (MDA), amyloidogenesis, activation of caspase 3 and 9, and the production of p53 and glutamate. It also inhibited the activity of Na^+/K^+ ATPase, glutamate dehydrogenase,

catalase, superoxide dismutase, and glutathione-S-transferase. The administration of Trévo revealed its antioxidant, anti-amyloidogenic, anti-excitotoxicity, and anticholinesterase properties as it prevented the biochemical changes induced by Cd toxicity in the brain of male Wistar rats. Our results supported the reported health benefits of Trévo as a good dietary supplement in preventing the toxic effects of poisonous substances, such as cadmium.

Keywords
Natural product; cadmium; neurotoxicity; apoptosis; beta-amyloid

1. Introduction

The usage of cadmium (Cd) and other toxic heavy metals has not ceased, especially with the development of solar power as an alternative form of energy [1], despite the efforts of regulatory agencies to minimize it.

Cd escapes into the environment through household appliances, paints, tobacco smoke, batteries, and agricultural products [1]. A report from the WHO showed that an average individual absorbs 0.4–0.5 mg of Cd every week [2]. The non-degradable nature of Cd, the inability of the body to excrete it, and a long half-life allow Cd to accumulate and persist in the body for a long time [3]. Cd mimics other molecules and enters the brain through blood circulation, and exhibits neurotoxic effects after it is absorbed from the lungs and the gastrointestinal tract (GIT) [4]. In the brain, Cd causes memory impairment, loss of cognitive functions, and other disabilities [5, 6]. Additionally, human exposure to Cd is implicated in the etiology of some neurodegenerative diseases [7, 8]. Cd has neurotoxic effects in various parts of the brain [9]. The mechanism of toxicity includes the cholinergic system, excitotoxicity, and apoptosis.

A constant feature in the findings of previous studies was the role of oxidative stress in Cd-induced neurotoxicity. Cd alters the redox status in the brain and leads to an increase in the generation of oxidants and inhibition of the antioxidant system [10]. Through oxidative stress, Cd can oxidize functional lipids and proteins on the membrane, further damaging the brain tissues. The high consumption of oxygen and the low levels of antioxidants in the brain make it more vulnerable to Cd poisoning compared to other organs [11].

Acetylcholine is a neurotransmitter that is affected by Cd poisoning and Cd-induced low concentrations of Ach in the synapse and astrocytes [12]. Anticholinergic effects can occur through an increase in the activity of acetylcholinesterase and/or blocking of the effect of the acetylcholine signal in the neurons [13, 14]. The anticholinergic effect of Cd is related to the memory deficit observed in patients exposed to Cd [15, 16].

The glutaminergic effect of Cd is associated with the ability of Cd to increase the concentration of glutamate in various parts of the brain. Glutamate, under physiological concentration, helps to excite the brain. However, under high concentrations, it can cause oxidative damage and stress to the antioxidant defense system, leading to neuronal cell death [2, 17].

Cadmium can initiate amyloidogenesis in the brain. An increase in the amount of amyloid in the brain due to Cd is associated with the ability of Cd to inhibit alpha-secretase and increase the levels

of amyloid precursor protein (APP). These alterations in the amyloidogenic process can increase the production of Amyloid beta plaques and decrease their elimination [6, 18, 19].

Another toxic effect of Cd is neuronal death caused by the activation of apoptotic proteins, such as caspases and p53. This process can be initiated by oxidative effects on membrane lipids and proteins. The p53 protein facilitates the clearance of damaged cells. However, high levels of p53 can attack normal cells. The p53 protein upregulates Bax and downregulates Bcl-2, leading to an increase in neuronal cell death [20]. Thus, the induction of p53 by Cd can alter several pathways leading to an increase in apoptosis [21, 22].

The renewed interest in the health benefits of natural products from medicinal plants has increased considerably in the last decades. Several regulatory agencies involved in drug production and consumption are encouraging the introduction of natural products in drug prescriptions [23]. Natural products have low side effects and are cheaper than most conventional drugs. They have several advantages over synthetic drugs, such as better antioxidant, anti-inflammatory, and stimulatory activities [24]. Trévo is a rich nutritional supplement composed of natural compounds extracted from fruits and plants that improve digestion, delay aging, and detoxify poisonous chemicals that are detrimental to various organs. It is a blend of several plant-based natural compounds produced in the USA under the trade name Trévo™ and is approved for use in Nigeria by the National Agency for Food and Drug Administration and Control (NAFDAC) with the registration number: A7–1020L and MCC 150821. Some herbs used for preparing Trévo™ include soursop, acai berry, camu-camu fruit, and ginseng. Additionally, it contains ascorbic acid, tocopherol, retinoic acid, essential amino acids, essential fatty acids, and nutrients (both micro and macro) that contribute to its health benefits. We had shown the protective effects of Trévo on the liver, kidney, and brain using various toxic chemicals in other studies [25, 26]. Here, we investigated the protective effects of Trévo against the biochemical alterations in the hippocampus following exposure to Cd.

2 Materials and Methods

2.1 Chemicals and Reagents

Cadmium chloride ($CdCl_2$), 1-Chloro-2,4-dinitrobenzene (CDNB), 5',5'-dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), acetylthiocholine iodide (AChI), nicotinamide adenosine dinucleotide (NAD), reduced nicotinamide adenine dinucleotide (NADH), and epinephrine were obtained from Sigma-Aldrich, USA. Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), hydrogen peroxide (H_2O_2), ethylenediamine tetra-acetic acid (EDTA), sodium chloride (NaCl), and sodium azide (NaN_3) were obtained from BDH (Poole, U.K.) and Hopkins & Williams (U.K.). Trèvo is a product of Trèvo™ LLC, Oklahoma City, USA. Other chemicals were of analytical grade.

2.2 Animal Care and Handling

In total, 30 adult male Wistar rats (170 ±10 g) were used for the experiment. They were purchased from the Central Animal House, University of Benin, Edo State, Nigeria, and used in this study. The animals were housed in well-ventilated cages and provided water and food ad libitum. The experimental design was approved by the Animal Research and Treatment (ART) ethics committee of the Federal University Otuoke, Nigeria (Code: ART2021005). The experiments were

conducted in the animal house of the Department of Biochemistry, Faculty of Science, Federal University Otuoke, between February and June 2021.

The rats were administered 2 mL/kg of Trévo™ [25] for five consecutive days before a single intraperitoneal administration of $CdCl_2$ (35 mg/kg, prepared by dissolving in normal saline) following the modified method of Khatani [16]. The dose administered was one-third of the median lethal dose (LD_{50}) of $CdCl_2$. The animals were sacrificed 24 h later after monitoring for any physiological changes.

In Group I, the animals were orally administered distilled water.

In Group II, the animals were intraperitoneally administered 35 mg/kg of $CdCl_2$ [16].

In Group III, the animals were orally administered 2 mL/kg of Trévo™ before the intraperitoneal administration of $CdCl_2$.

2.2.1 Processing of the Brain

After sacrificing the rats under light anesthesia, their brains were excised. It was then rinsed with ice-cold rinsing buffer (1.5% KCl), weighed, and the hippocampus was carefully separated from the rest of the brain and homogenized in 0.25 M sucrose. The homogenate was centrifuged at 4 °C for 10 min using a cold ultracentrifuge that was set at 15,000 rpm (7,500 g) (IEC: CENTRA-GP8R, DJB Labcare, UK model.). After centrifugation, the clear supernatant was pipetted and stored in the freezer for biochemical assays.

2.3 Biochemical Assay

2.3.1 Estimation of Acetylcholinesterase (AChE) Activity and β-amyloid Concentration

The activity of AChE was measured in the hippocampus of the rat brain using a spectrophotometric method following the instructions provided in the kit manuals. The concentration of *β*-amyloid in the hippocampus was quantified using an ELISA kit (Catalog Number: CSB-EL001950RA) and following the guidelines in the manuals provided by Ray Biotech Inc. (Norcross, GA, USA).

2.3.2 Determination of Glutamate, Glutamate Dehydrogenase (GD), and Sodium/Potassium ATPase (Na^+/K^+ ATPase)

The concentration of glutamate and the activities of GD and Na^+/K^+ ATPase were measured in the hippocampus using a spectrophotometric method following the instructions provided in the kit manuals. All assay kits were purchased from Cell Signaling Technologies, Danvers, USA.

2.3.3 Estimating the Concentration of p53 and the Activities of Caspase 3 and 9

The tumor suppressor gene p53 in the brain homogenates was evaluated following the method described by Yang et al. [27] using Enzyme-linked Immunosorbent Assay (ELISA) kits (Catalog Number. CSB-E08334 h). The activities of caspase 3 and 9 were estimated using an ELISA kit (Catalog Number. CSB-E08862 h), following the instructions provided in the kit manual supplied by Calbiochem.

2.3.4 Determination of Malondialdehyde (MDA) and Reduced Glutathione (GSH) Concentration

The concentration of MDA generated was measured following the method described by Varshney and Kale [28]. The reaction mixture consisted of 0.4 mL of sample, 1.6 mL of TRIS-KCl buffer, 0.5 mL of 30% TCA, and 0.5 mL of TBA. The mixture was boiled at 80 °C for 45 min. After it was cooled down, the mixture was centrifuged, and the absorbance of the clear supernatant was recorded at 532 nm. The concentration of GSH was measured following the method described by Jollow et al. [29]. Briefly, 0.2 mL of the sample was diluted with 1.8 mL of distilled water and 3 mL of sulfosalicylic acid. The solution was centrifuged at 3,000 g for 10 min to obtain a clear supernatant. Then, 0.5 mL of the clear supernatant was added to DTNB and phosphate buffer (0.1 M, pH = 7.4). Finally, the absorbance was recorded at 412 nm.

2.3.5 Determination of Catalase (CAT) and Superoxide Dismutase (SOD) Activities and Estimation of Glutathione-S-Transferase (GST) Activity

The activities of CAT and SOD were measured following the method described by Aebi [30] and Misra and Frivrovich [31], respectively. For SOD, 1 mL of the sample was diluted with 9 mL of distilled water. Then, 2.5 mL of the diluted sample was mixed with 2.5 mL of carbonate buffer (0.075 M, pH = 10.2) inside the cuvette before adding 0.3 mL of adrenaline. The increase in absorbance of the reacting mixture was recorded every 30 s for 150 s. The activity of SOD was expressed as the amount of SOD that caused 50% inhibition of adrenaline oxidation per minute. The determination of the activity of CAT in the sample involved mixing 2 mL of H_2O_2 solution (0.8 mmol), 2.5 mL phosphate buffer (0.1 M, pH = 7.4), and 0.5 mL of the sample. The mixture was allowed to react at room temperature, after which 1 mL of the mixture was pipetted into a test tube containing 2 mL of dichromate/acetic acid reagent at 60 s intervals for 180 s. The change in the color stabilized after incubating for 10 min at 100 °C. The cooled solution was read at 520 nm. The activity of CAT was expressed as U/mg protein. The activity of GST was evaluated following the method described by Habig et al. [32]. Briefly, 30 µL of the sample was pipetted into a cuvette containing 30 µL of GSH, 150 µL of CDNB, and 2.79 mL phosphate buffer (0.1 M, pH = 6.5). The mixture was allowed to stabilize inside the cuvette before recording the absorbance every 60 s for 5 min against a reagent blank. The activity of GST was expressed as ⌈mol of CDNB conjugated/min/mg protein.

2.4 Statistical Analysis

The data obtained from the experiment were analyzed using GraphPad Prism 6.01 for windows by performing a one-way analysis of variance (ANOVA) and Duncan's multiple range tests. Different symbols indicate significant differences between groups at $P < 0.05$. The results are expressed as the mean ±standard deviation of 10 animals per group.

3. Results

3.1 Concentration of β-amyloid and Activity of AChE

To evaluate the protective effect of Trévo against some biochemical markers associated with neurodegenerative diseases, the concentrations of β-amyloid (a biomarker of Alzheimer's disease) and acetylcholinesterase were analyzed (see Figures 1A and 1B). The results showed that the activity

of AchE and the concentration of β-amyloid were significantly higher in the animals administered $CdCl_2$ compared to that in the animals of the control group ($P < 0.001$); Trévo reversed the effects of $CdCl_2$ ($P < 0.001$).

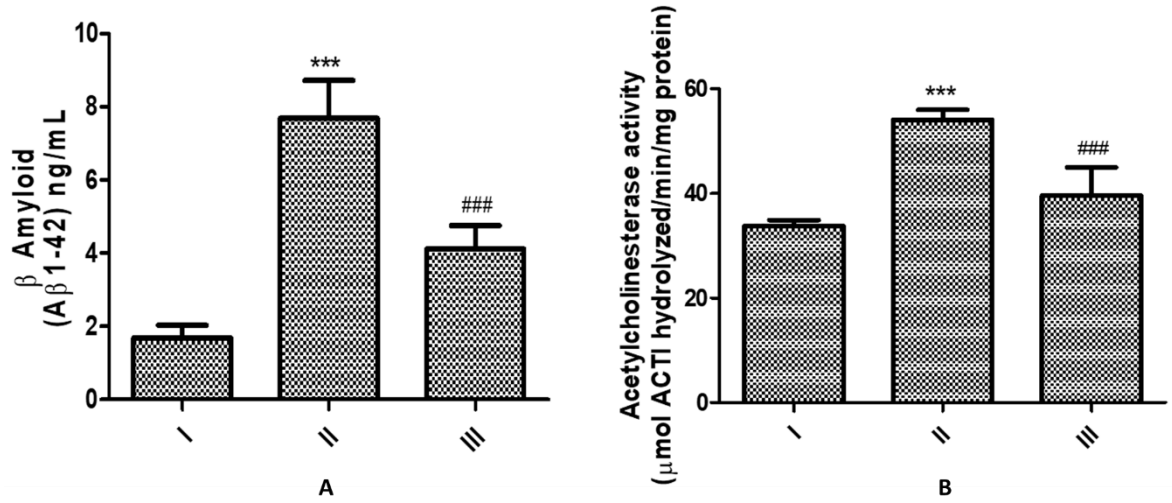

Figure 1 A: The protective effect of Trévo against cadmium-induced β-amyloid generation in the rat brain. **B**: The activity of AchE in the rat brain following the administration of Trévo and cadmium chloride ($CdCl_2$). The results are expressed as the mean ±SD (n = 10). Statistically significant differences: $^{***}P < 0.001$ = Control vs. $CdCl_2$; $^{###}P < 0.001$ = $CdCl_2$ vs. 2 mL/kg Trévo + $CdCl_2$. **I** = Control; **II** = 35 mg/kg $CdCl_2$; **III** = 2 mL/kg Trévo + $CdCl_2$.

3.2 Concentration of Glutamate and Activities of Glutamate Dehydrogenase and Na^+/K^+ ATPase

The effect of $CdCl_2$ and Trévo on glutamate (excitatory amino acid) concentration, activities of glutamate dehydrogenase, and Na^+/K^+ ATPase are presented in Figures 2A, 2B, and 2C, respectively. The results showed that $CdCl_2$ caused a significant increase in the glutamate concentration ($P < 0.001$). Treatment with Trévo reversed the effect, but the difference in the glutamate concentration before and after the administration of Trévo was not significant ($P > 0.05$). The results also showed that $CdCl_2$ caused a significant decrease in the activities of GD ($P < 0.05$) and Na^+/K^+ ATPase ($P < 0.001$), and treatment with Trévo had a mild effect on reversing the toxic effects of $CdCl_2$.

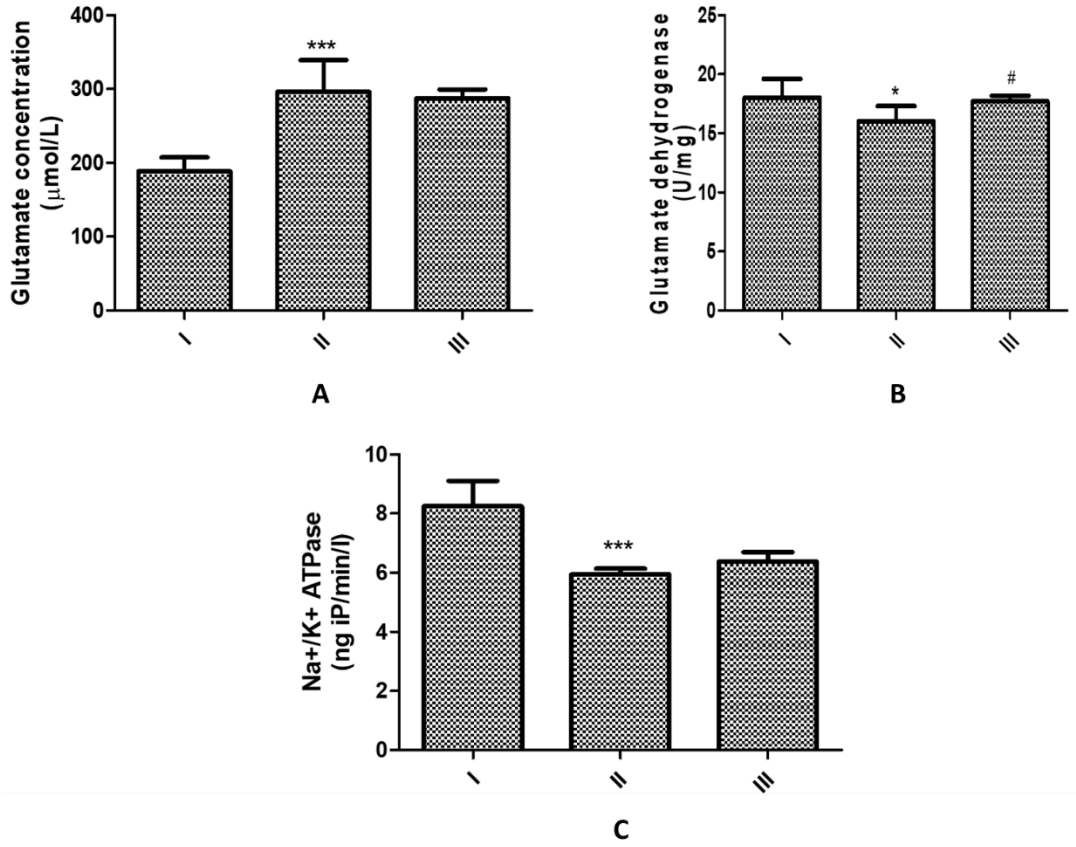

Figure 2 A: The concentration of glutamate in the rat brain following the administration of Trévo and $CdCl_2$. **B**: The activity of glutamate dehydrogenase in the rat brain following the administration of Trévo and $CdCl_2$. **C**: The activity of Na^+/K^+ ATPase in the rat brain following the administration of Trévo and $CdCl_2$. The results are shown as the mean ±SD (n = 10). Statistically significant differences: $^{*}P < 0.05$ = Control vs. $CdCl_2$; $^{\#}P < 0.05$ = $CdCl_2$ vs. 2 mL/kg Trévo + $CdCl_2$; $^{***}P < 0.001$ = Control vs. $CdCl_2$. **I** = Control; **II** = 35 mg/kg $CdCl_2$; **III** = 2 mL/kg Trévo + $CdCl_2$

3.3 Trévo Reversed the Alteration in Apoptotic Proteins Induced by $CdCl_2$

The effect of $CdCl_2$ and Trévo on the concentrations of p53, caspase-3, and caspase–9 are shown in Figures 3A, 3B, and 3C, respectively. The results showed that $CdCl_2$ caused a significant increase in the concentrations of p53 ($P < 0.001$), caspase-3 ($P < 0.01$), and caspase–9 ($P < 0.001$). Treatment with Trévo decreased the concentrations of p53 ($P < 0.001$) and caspase-9 ($P < 0.001$), but it did not affect the concentration of caspase-3.

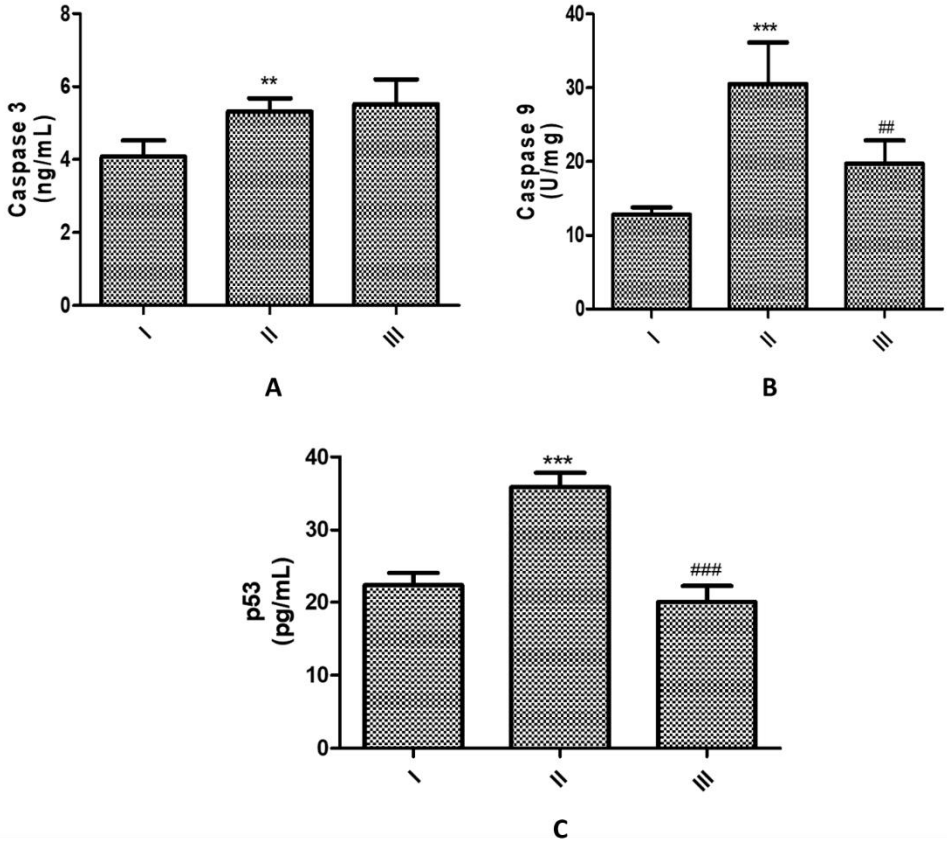

Figure 3 A: The concentration of p53 in the rat brain following the administration of Trévo and $CdCl_2$. **B**: The concentration of caspase 3 in the rat brain following the administration of Trévo and $CdCl_2$. **C**: The concentration of caspase-9 in the rat brain following the administration of Trévo and $CdCl_2$. The results are expressed as the mean ±SD (n = 10). Statistically significant differences: $^{**}P < 0.01$ = Control vs. $CdCl_2$; $^{***}P < 0.001$ = Control vs. $CdCl_2$; $^{\#\#}P < 0.01$ = $CdCl_2$ vs. 2 mL/kg Trévo + $CdCl_2$; $^{\#\#\#}P < 0.001$ = $CdCl_2$ vs. 2 mL/kg Trévo + $CdCl_2$. **I** = Control; **II** = 35 mg/kg $CdCl_2$; **III** = 2 mL/kg Trévo + $CdCl_2$.

3.4 Redox Status

We investigated the redox status in the brain by measuring the concentrations of MDA and GSH, as shown in Figures 4A and 4B, respectively. The results showed that the administration of $CdCl_2$ caused a significant increase in the concentration of MDA ($P < 0.05$), a decrease in the concentration of GSH ($P < 0.001$). Treatment with Trévo significantly reversed the oxidative effects of $CdCl_2$.

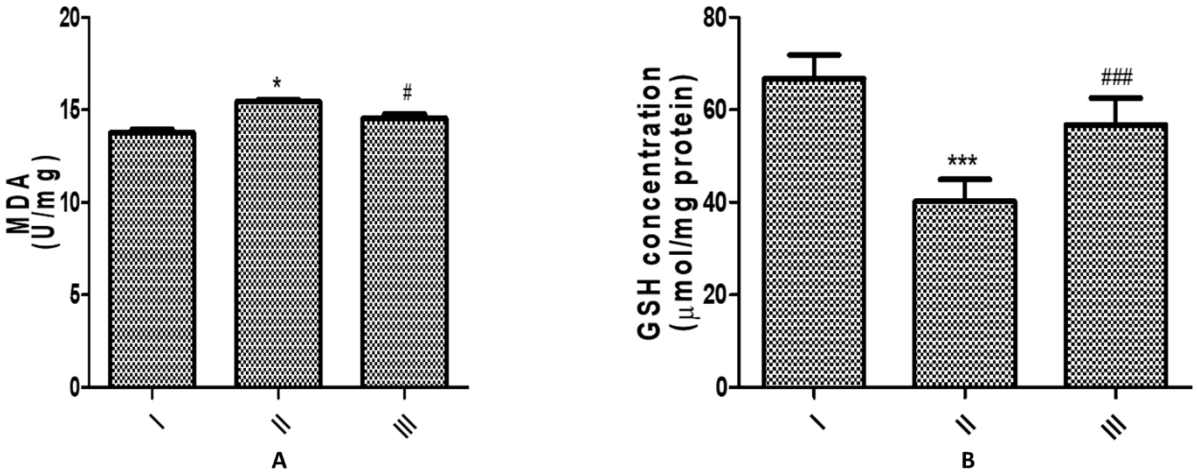

Figure 4 A: The effect of Trévo on $CdCl_2$-induced malondialdehyde (MDA) generation in the rat brain. **4 B**: The effect of Trévo and $CdCl_2$ on reduced glutathione (GSH) in the rat brain. The results are expressed as the mean ±SD (n = 10). Statistically significant differences: $^{*}P < 0.05$ = Control vs. $CdCl_2$; $^{***}P < 0.001$ = Control vs. $CdCl_2$; $^{\#}P < 0.05$ = $CdCl_2$ vs. 2 mL/kg Trévo + $CdCl_2$; $^{\#\#\#}P < 0.001$ = $CdCl_2$ vs. 2 mL/kg Trévo + $CdCl_2$. **I** = Control; **II** = 35 mg/kg $CdCl_2$; **III** = 2 mL/kg Trévo + $CdCl_2$.

3.5 Antioxidant and Drug Metabolizing Enzymes

The activity of SOD, CAT, and glutathione-S-transferase (GST), were measured in the brain, and the results are presented in Figures 5A, 5B, and 5C, respectively. $CdCl_2$ caused a significant decrease in the activities of SOD, CAT, and GST ($P < 0.001$), which was reversed by the pre-administration of Trévo ($P < 0.001$).

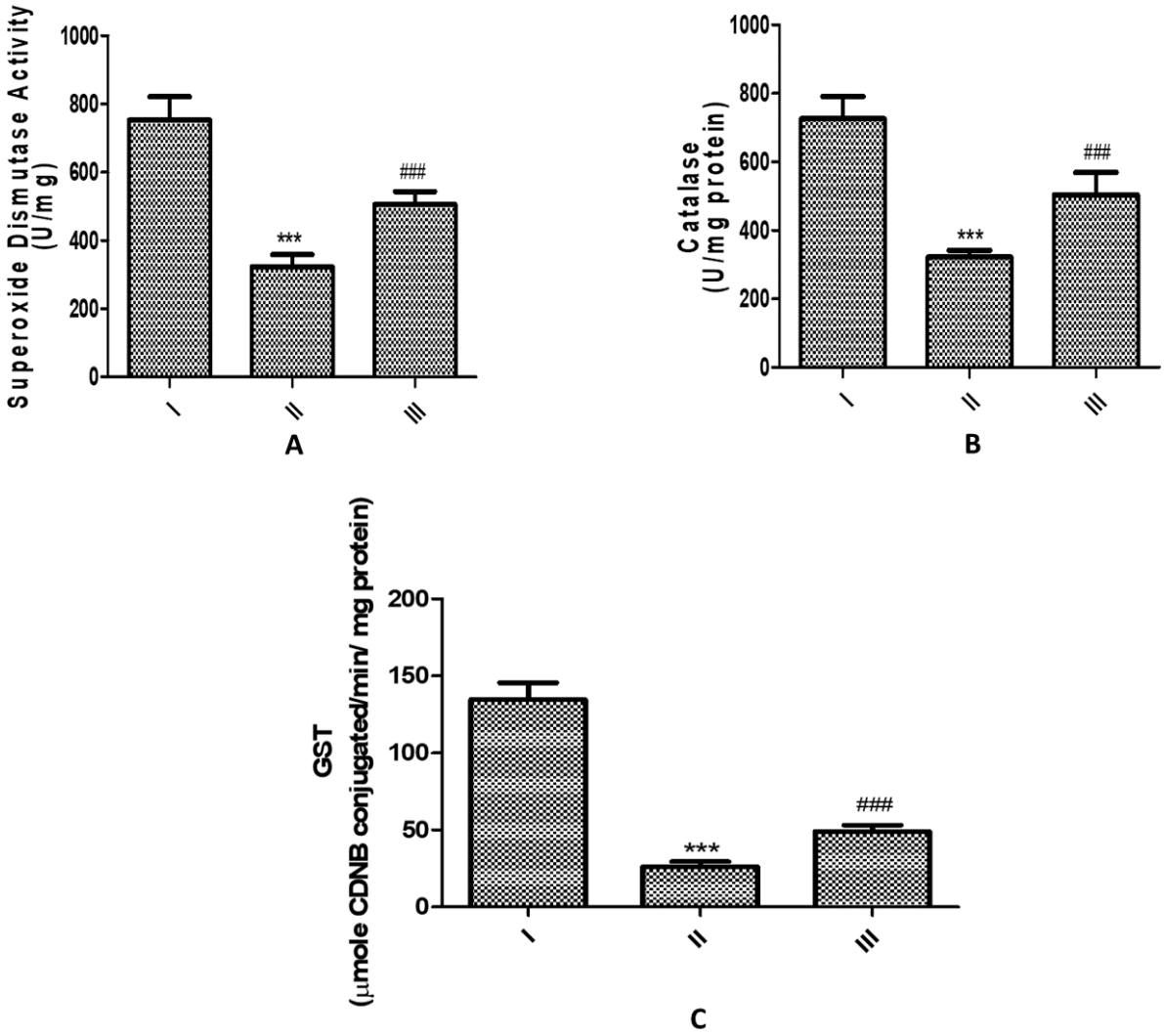

Figure 5 A: The effect of Trévo and $CdCl_2$ on the activity of superoxide dismutase (SOD) in the rat brain. **B**: The effect of Trévo and $CdCl_2$ on the activity of catalase in the rat brain. **C**: The effect of Trévo and $CdCl_2$ on the activity of glutathione-S-transferase (GST) in the rat brain. The results are expressed as the mean ±SD (n = 10). Statistically significant differences: $^{***}P < 0.001$ = Control vs. $CdCl_2$; $^{###}P < 0.001$ = $CdCl_2$ vs. 2 mL/kg Trévo + $CdCl_2$. **I** = Control; **II** = 35 mg/kg $CdCl_2$; **III** = 2 mL/kg Trévo + $CdCl_2$. CDNB = 1-Chloro-2,4-dinitrobenzene.

4. Discussion

A major factor that makes the brain prone to oxidative damage is its low antioxidant system compared to other organs. The neurotoxic effect of cadmium (Cd) was shown in many studies [33-36]. Its mechanism of neurotoxicity is associated with an increase in the generation of ROS, resulting in oxidative stress. To confirm the neurotoxic effects of Cd, we evaluated the effect of Cd on the redox status in the brain of male Wistar rats. The concentrations of MDA and GSH and the enzyme activities of SOD, CAT, and GST were measured. The results showed that exposure to Cd (35 mg/kg) significantly altered the redox status of the brain, which led to oxidative stress. Cadmium suppressed the enzymatic antioxidant system and reduced the activities of CAT and SOD, which

caused an increase in the MDA concentration and a decrease in the GSH concentration. The oxidative effect of the accumulated Cd in the brain is due to its lipophilic nature, which enables it to cross the blood-brain barrier [37]. In the brain, Cd shows a high affinity for the sulfhydryl (-SH) group of GSH, leading to its reduction. Additionally, the binding of Cd to the sulfhydryl group in the antioxidant enzyme can also inhibit or suppress their activities [38, 39]. Treatment with Trévo significantly prevented the alteration in the redox status by inhibiting the process that generates MDA and prevented the destruction of GSH and antioxidant enzymes by Cd. The protective effect of Trévo might be due to the presence of polyphenols and flavonoids in Trévo.

The results also showed that Cd increased the activity of AChE in the hippocampus. The increase in the activity of AChE by Cd might be due to the ability of Cd to alter the lipid bilayer of the membrane. AChE catalyzes the breakdown of acetylcholine into choline and acetate. Thus, an increase in the activity of AChE decreases the stimulation of acetylcholine receptors in the hippocampus, leading to memory impairment [40]. Our findings suggested that Cd impairs cognitive functions by increasing the activity of AChE.

Glutamate is an excitatory amino acid that plays an important role in neurodegenerative diseases [41, 42]. We found that Cd increased the concentration of glutamate in the hippocampus. Additionally, Cd altered some of the enzymes involved in glutamate breakdown and transportation from the cells (glutamate dehydrogenase and Na^+/K^+ ATPase). The inhibition of these enzymes by Cd can further increase glutamate concentration in the hippocampus, leading to their deterioration. Our results indicated that Cd can stimulate excitatory neurotransmitters, such as glutamate, and impair synaptic and neurocognitive functions [43].

An increase in the concentration of beta-amyloid in the brain is an important indicator for the development of AD. The generation of beta-amyloid signals the presence of toxicants that are associated with AD [44]. ROS can induce the formation of Aβ in the brain, and Aβ can also increase the generation of ROS and MDA in the brain [45]. Our results showed that Cd increased the concentration of Aβ in the hippocampus. This was similar to the findings of other investigators [46, 47] and also confirmed the effect of Cd on the development of neurodegenerative diseases. The neurodegenerative effect of Aβ is strongly associated with apoptosis [16]. In this study, we examined the effect of Cd on the concentration of p53, caspase-3, and caspase–9 in the hippocampus. The tumor suppressor protein p53 initiates cell death. The activation of caspase-9 initiates the activation of other caspases, leading to a cascade of events that eventually cause cell death, while caspase-3 is involved in the formation of apoptotic bodies and plays a role in cell death. Our results showed that Cd increased the concentration of p53 and the activities of caspase-3 and caspase–9 in the hippocampus. Caspase-3 and caspase–9 were found to play a role in the development of neurodegenerative diseases and other forms of brain-related disorders [47, 48]. Thus, our results indicated that Cd promotes apoptosis by increasing the levels of p53 and activating caspases. A strategy for developing drugs for treating neurodegenerative diseases is to test their inhibitory effect against caspases [49]. The mechanism of Cd neurotoxicity involves the interplay of ROS, Aβ, glutamate, and apoptosis. The alterations of these molecules and processes lead to the injury and death of cells in the hippocampus.

Treatment with Trévo had different effects on the biochemical toxicity of Cd in the hippocampus of male Wistar rats. Our results showed that Trévo can reverse the oxidative effect of Cd in the hippocampus. The GSH concentration and the SOD, CAT, and GST activities increased significantly. The antioxidant activity of Trévo against Cd neurotoxicity is expected as Trévo is a blend of

polyphenol-rich plant products. Polyphenols present in Trévo include pomegranate, lycopene, β-carotene, ascorbic acid, and omega-3 and omega-6 fatty acids. These antioxidant phytochemicals can prevent, delay, or reverse neurological disorders [33, 50-56]. They probably also contribute to the antioxidant properties of Trévo. The anticholinesterase and antiglutamate activities of Trévo were also observed in the hippocampus following exposure to Cd. Polyphenols can inhibit AChE activity and prolong the neurostimulatory effect of AChE in the hippocampus [57, 58]. The drugs that are used for treating AD inhibit AChE. The presence of bioactive phytochemicals, minerals, and vitamins in Trévo might be responsible for the ameliorative effect against Cd neurotoxicity. Our results also showed that Trévo inhibited the formation of Aβ and p53 and the activation of caspase-9 in the hippocampus, indicating its ability as an anti-apoptotic drug against Cd-induced neurotoxicity.

Nutrients play an important role in intercepting the toxic effects of poisonous metals. They can also modulate the synthesis of neurotransmitters and the intercellular response of cells. Some micronutrients often act as co-factors or ligands in the enzymes and proteins involved in the synthesis and efficient functioning of neurotransmitters. Thus, the absence of these nutrients can further exacerbate the toxic effects of Cd. Vitamin and mineral deficiencies can also influence the development of neurodegenerative disorders [59]. Dietary supplements containing some of these micronutrients are effective in combating the toxic effects of heavy metals, such as Cd, Hg, and Pb, in the brain [60]. Plant-rich phenolic compounds can also slow down aging through their antioxidant and anti-apoptotic effects; their mechanism of action includes inhibition of the activities of caspases, which in turn can arrest apoptosis [61, 62]. Trévo^{TM} is a nutritional supplement that is rich in all these micronutrients, natural products, essential oils, and nutrients that contribute to its health-promoting effects.

This study had some limitations. Neurobehavioral studies, such as those on motor and memory deficits, were not conducted to confirm the effects of Trévo on physiological parameters. Also, animals were pretreated with Trévo before exposure to Cd to evaluate the chelating and detoxification effects of Trévo. We did not measure the concentration of Cd in the brain. Future studies should involve posttreatment of Trévo and analyze the effects of Trévo on other regions of the brain following exposure to Cd. The studies should also assess the effects of Trévo on motor and memory deficits.

5. Conclusion

To summarize, Trévo^{TM} can mitigate the neurotoxicity of Cd in male Wistar rats. The neuroprotective effect of Trévo^{TM} is due to the presence of rich natural antioxidants, essential nutrients, and vitamins with confirmed health benefits in humans. We also found that Trévo^{TM} can prevent some biochemical alterations observed in patients suffering from neurodegenerative diseases.

Author Contributions

All the authors were involved in conceptualization, methodology, formal analysis, writing and editing of the manuscript.

Competing Interests

The authors declare no conflict of interest.

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