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Gallic acid abates cadmium chloride toxicity via alteration of neurotransmitters and modulation of inflammatory markers in Wistar rats

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Cadmium is a highly neurotoxic heavy metal that disrupts membranes and causes oxidative stress in the brain. The study aimed to investigate the neuroprotective effect of gallic acid on oxidative damage in the brains of Wistar rats exposed to cadmium chloride ($CdCl_2$). Male Wistar rats were divided into four groups of five rats each. Group 1 was administered distilled water only throughout the study. Throughout the study, Group 2 received $CdCl_2$ alone (5 mg/kg b.w./day), Group 3 received gallic acid (20 mg/kg b.w./day), and Group 4 received $CdCl_2$ + gallic acid (20 mg/kg). Treatments were oral with distilled water as a vehicle. The study lasted 21 days. In the brain, the activities of cholinesterase and antioxidant enzymes were evaluated, as well as the levels of reduced glutathione, malondialdehyde, neurotransmitters, Na+/K+ ATPase, myeloperoxidase activity, nitric oxide, and interleukin-6. $CdCl_2$-induced brain impairments in experimental animals and gallic acid prevents the following $CdCl_2$-induced activities: inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), elevated neurotransmitters (serotonin and dopamine), decreased antioxidant enzymes (superoxide dismutase, catalase), decreased glutathione, Na+/K+ ATPases, and increased MDA and neuroinflammatory markers (myeloperoxidase (MPO), nitric oxide, and interleukin-6 in the brain of experimental rats exposed to $CdCl_2$ ($p < 0.05$). Taken together, the neuroprotective effects of gallic acid on $CdCl_2$-induced toxicity in the brains of rats suggest its potent antioxidant and neurotherapeutic properties.

Cadmium (Cd) is a toxic heavy metal that has a negative impact on human and animal cellular and metabolic systems. Cd levels in brain tissue are always high in rats that have been intoxicated with the metal (at dosage roughly 700 times higher than the control values). Cadmium salts is one of the most toxic pollutants found in the environment. Because Cd is not broken down, the danger of human exposure to Cd via food chain pollution is constantly increasing[1]. Exposure to cadmium occurs primarily through foods such as cereals and vegetables, implying that exposure is constant. The rise in Cd contamination is a major public health concern around the world[2]. Long-term exposure to Cd is well known to cause toxic side effects in numerous organ systems, like the cardiovascular, brain, and immune/hemopoietic[3,4]. Cd has the potential to cause neurotoxicity, resulting in an extensive range of clinical entities such as neurological disturbances and changes in normal brain neurochemistry[5]. Cd has been shown to promote lipid peroxidation (LPO) by increasing free radicals[6]. Cd toxicity has been linked to the production of reactive oxygen species (ROS) and the depletion of antiradicals[7]. The capability of Cd to cause oxidative stress in brain cells has been described as ROS generation after Cd intoxication with mitochondrial sites, resulting in the breakdown of mitochondrial potential and a subsequent decrease in intracellular glutathione levels[8]. Due to its high rate of oxygen utilization, abundance of polyunsaturated fatty acids, weak antioxidant

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defense, and high concentration of transition metals like copper and iron in some areas, brain tissue is highly susceptible to lipid peroxidation[9]. Loss of membrane-bound ATPase activity may occur as a result of the brain membranes' increased sensitivity to LPO[10]. Lipid-dependent membrane-bound enzymes known as ATPases play a role in active transport, preservation of cell homeostasis, and neurotransmission[11]. Cadmium modifies the activities of oxidative stress-neutralizing enzymes, causing disruptions in brain metabolism and contributing to cadmium's neurotoxic effect. Cadmium causes changes in the structural integrity of lipids and has indirect effects on membrane-bound enzymes by increasing the production of free radicals in the brain and interfering with the antioxidant defense system[12]. The ability of Cd to cross the blood–brain barrier may explain its accumulation in brain tissue[13]. Reactive oxygen species (ROS) production is a normal process in metabolism. Excessive ROS production, not compensated by antioxidants, cause oxidative stress[14]. Presently, a broad investigation is being conducted to assess the preventive effects of many natural antioxidants on metal-induced toxicities[15,16]. Antioxidants are gaining increasing attention in the treatment of diseases related to oxidative stress as well as being probable therapeutic agents for a variety of disorders. Antioxidants can mitigate the cadmium-induced reduction in ATPase activity and the elevation of oxidative injury[17].

One of the well-studied antioxidant agents is gallic acid. Gallic acid is a common plant metabolite that has the structure of trihydroxybenzoic acid and several hydrogen atoms in its phenolic structure that easily delocalize free radicals[18,19]. Its structure explains why it has strong antioxidant properties, indicating that it can protect tissues and organs from oxidative stress[18,20]. Gallic acid has previously been reported to be a common component of various foods and herbal drugs[21]. GA protects the brain by increasing antioxidant enzymes and decreasing inflammation[22,23]. Gallic acid can reduce the incidence of inflammation-related diseases, including cancer[24,25], cardiovascular disease[26,27], liver disease[28,29], inflammation[30,31], and neurodegenerative diseases. Gallic acid can inhibit the inflammatory process by eliminating superoxide anions, inhibiting the release and activity of myeloperoxidase (MPO), and possibly participating in the accumulation of active NADPH-oxidase[31]. This suggests that gallic acid is a promising chemical agent with a variety of chemotherapeutic properties. Given gallic acid's antioxidant and anti-inflammatory properties, it is worth considering gallic acid as a potential preventive agent against cadmium-induced oxidative stress in the brain. The purpose of the study was to examine the protective role of gallic acid against oxidative damage caused by $CdCl_2$ in the brains of male Wistar rats. As a result, we investigated the effects of gallic acid on the activities of AChE, BChE, and Na+/K+ ATPase. Furthermore, we examine the protective abilities of gallic acid on antioxidant enzymes (catalase and SOD) as well as GSH levels, markers of oxidative stress (TBA Reactive Substances (TBARS)), neurotransmitters (serotonin and dopamine levels), and inflammatory markers (MPO, nitric oxide (NO), and IL-6) in the brains of rats treated with $CdCl_2$ to give more scientific evidence on the protective role of gallic acid.

Results

Gallic acid enhanced antioxidant status in cadmium chloride-induced Wistar rats. Figure 1 shows the effects of a 21-day exposure to $CdCl_2$ and gallic acid on SOD, catalase, and GSH in the cerebrum of Wistar rats. $CdCl_2$ alone was found to significantly ($p < 0.05$) reduce SOD, catalase, and GSH levels. On the other hand, $CdCl_2$, markedly ($p < 0.05$) raised MDA levels in the cerebrum of experimental rats. However, compared to rats given only $CdCl_2$, co-administration of $CdCl_2$ and gallic acid at a dose of 20 mg/kg bwt markedly ($p < 0.05$) raised the activities of SOD and catalase as well as GSH level.

Gallic acid reversed the inhibition of brain activities of acetylcholinesterase and butyrylcholinesterase in rats administered with $CdCl_2$. Figure 2 shows the impact of gallic acid on the brain activities of AChE and BChE in the cerebrum of rats given $CdCl_2$. Compared to control rats, $CdCl_2$ markedly ($p < 0.05$) inhibited AChE and BChE activities in the cerebrum of Wistar rats (Fig. 2). However, the activities of both enzymes were significantly decreased ($p < 0.05$) when $CdCl_2$ and gallic acid were administered together.

Gallic acid reverses brain levels of serotonin and dopamine in rats exposed to $CdCl_2$. The effect of gallic acid on brain neurotransmitter levels of serotonin (Fig. 3) and dopamine (Fig. 4) in the cerebrum of rats administered $CdCl_2$. $CdCl_2$ alone significantly ($p < 0.05$) reduced serotonin levels and increased dopamine levels in the brains of Wistar rats compared to control rats. On the contrary, the co-administration of gallic acid with $CdCl_2$ significantly ($p < 0.05$) prevent such alterations in the level of neurotransmitters. Furthermore, in rats administered only gallic acid, serotonin levels increased and dopamine decreased compared to $CdCl_2$ alone ($p < 0.05$).

Gallic acid protected against $CdCl_2$-induced neuro inflammation. The gallic acid effects on MPO, NO, and interleukin-6 (IL-6) brain activity in the cerebrum of rats receiving $CdCl_2$ are shown in Figs. 5, 6, and 7. $CdCl_2$ alone significantly ($p < 0.05$) raised the levels of MPO (Fig. 5), NO (Fig. 6), and IL-6 (Fig. 7) in comparison to the control. Co-administration of $CdCl_2$ and gallic acid decreased NO and IL-6 levels as well as MPO activity in comparison to rats exposed to $CdCl_2$ alone.

Effect of gallic acid on membrane bound ATPases in rat brain. Changes in ATPase enzyme activity (Na^+/K^+-ATPase) in the cerebrum of control and experimental rats are depicted in Fig. 8. Compared to control rats, $CdCl_2$ alone rats had a significant decrease ($p < 0.05$) in the activity of the ATPase enzyme. Compared to cadmium alone, gallic acid co-administration significantly increased ($p < 0.05$) the activity of ATPase enzymes in the brain.

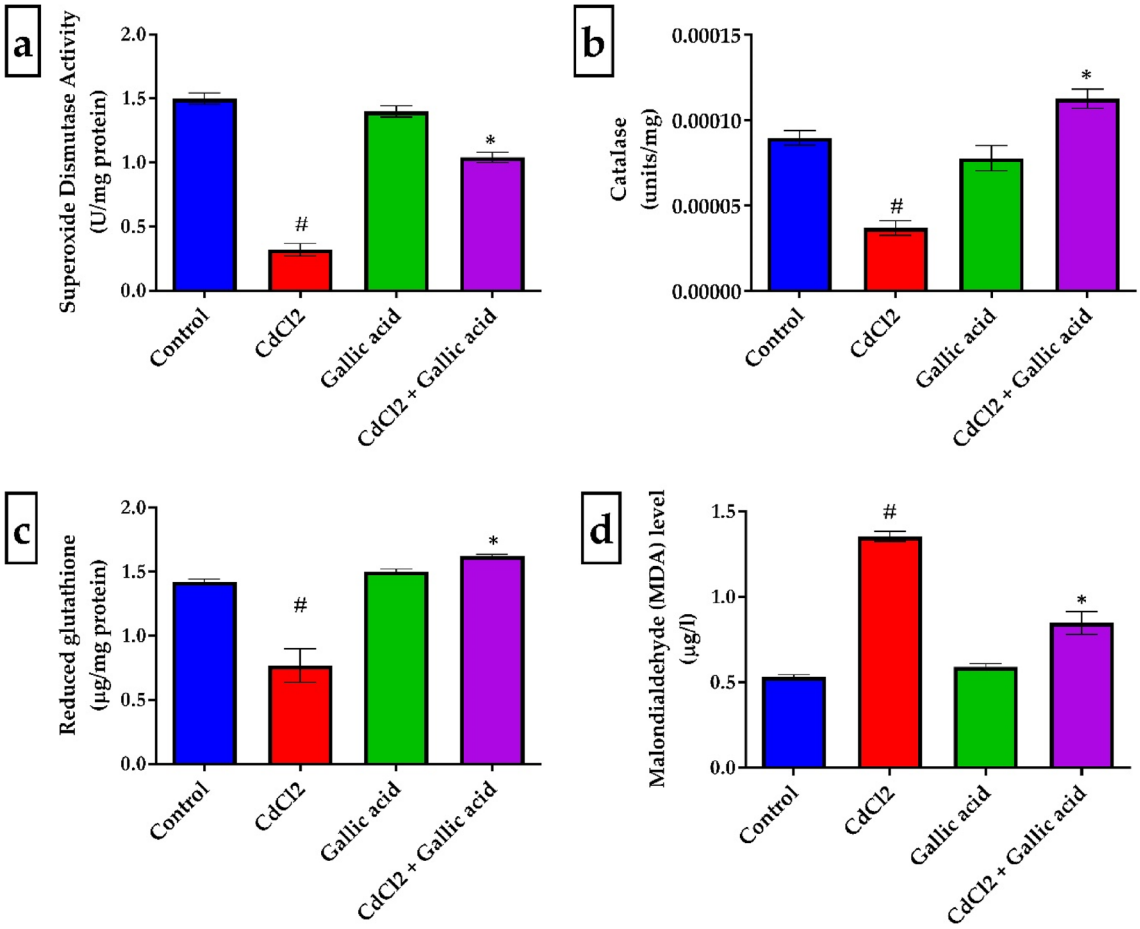

Figure 1. Brain cerebrum levels of antioxidants and MDA in control and treated groups. (**a**) SOD, (**b**) CAT, (**c**) GSH, and (**d**) MDA between the control and treated groups. The data is represented as mean ± SD (n = 5). #Significant at $p < 0.05$ compared to the negative control; *Significant at $p < 0.05$ compared to the $CdCl_2$ alone.

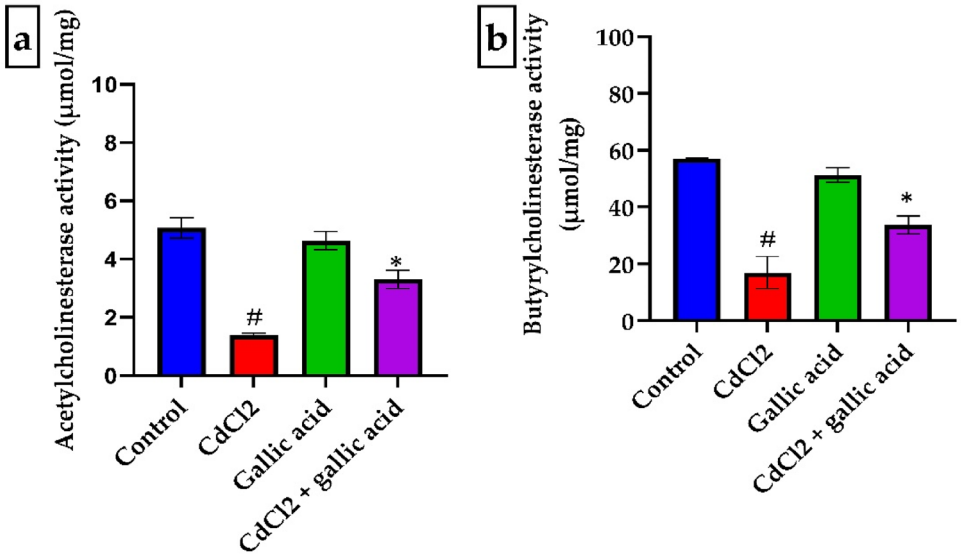

Figure 2. Gallic acid reverses the inhibition of (**a**) acetylcholinesterase (AChE) and (**b**) butyrylcholinesterase (BChE) of rats administered with distilled water, $CdCl_2$ alone, gallic acid alone, and $CdCl_2$ + gallic acid (20 mg/kg). The data is represented as mean ± SD (n = 5). #Significant at $p < 0.05$ compared to the control; *Significant at $p < 0.05$ compared to the $CdCl_2$ only group.

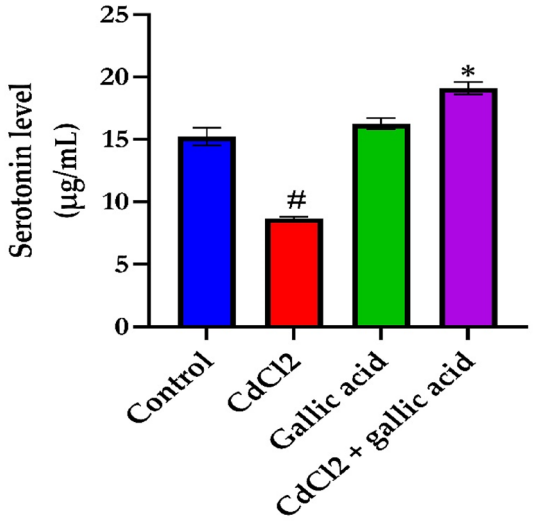

Figure 3. Gallic acid reverses the levels of serotonin in the cerebrum of rats administered with distilled water, $CdCl_2$ alone, gallic acid alone, and $CdCl_2$ + gallic acid (20 mg/kg). The data is represented as mean ± SD (n = 5). #Significant at $p < 0.05$ compared to the control; *Significant at $p < 0.05$ compared to the $CdCl_2$ only group.

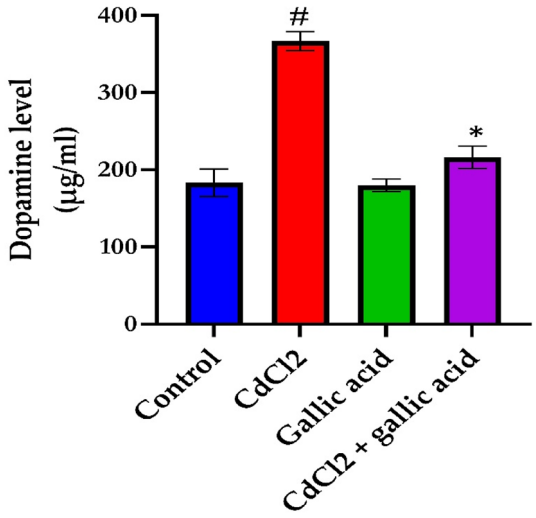

Figure 4. Gallic acid reverses the levels of dopamine in the cerebrum of rats administered with distilled water, $CdCl_2$ alone, gallic acid alone, and $CdCl_2$ + gallic acid (20 mg/kg). The data is represented as mean ± SD (n = 5). #Significant at $p < 0.05$ compared to the control; *Significant at $p < 0.05$ compared to the $CdCl_2$ only group.

Impact of gallic acid on brain of rats with $CdCl_2$-induced neurotoxicity. Figure 9 shows the histological studies of the cerebrum of experimental animals after the administration of $CdCl_2$ + gallic acid. In contrast to the normal architecture displayed by the cerebrum of normal and gallic acid control rats, the cerebrum of $CdCl_2$-induced rats had severe vacuolation of purkinje cell layer, optical empty spaces due to cell necrosis, severe separation of purkinje cell layer from granular layer, severe hemorrhage, and cell degeneration. The co-administration of $CdCl_2$ with gallic acid decreased the occurrence of these changes in the cerebrum of rats and revealed near-normal architecture comparable to the $CdCl_2$ alone rats.

Discussion

According to recent research, Cd exposure in Wistar rats weakening antioxidant defense mechanisms, which messes up cellular redox and causes oxidative stress. During oxidative stress, free radicals are produced in large quantities, causing a reduction in antioxidant enzymes like superoxide dismutase and catalase[32], which may have helped control ROS in the brain[33]. However, the brain can suffer harm if ROS is generated by interacting with the lipids, carbohydrates, proteins, and DNA present in the brain[31]. This study suggests that $CdCl_2$ toxicity is caused by oxidative stress, which interacts with various brain cells and tissues. As a result, $CdCl_2$-induced oxidative stress in rat brains, leading to decreased SOD and catalase activities, which is a sign of oxidative damage.

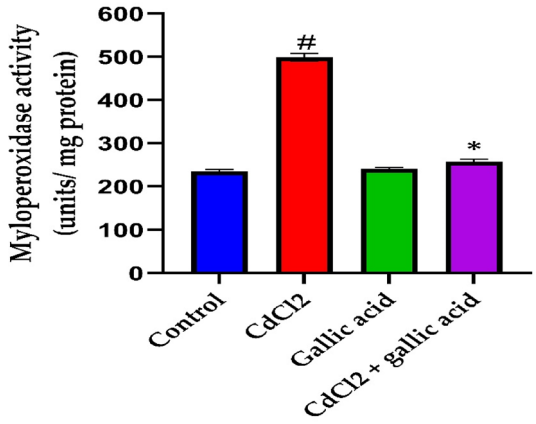

Figure 5. Gallic acid reverses brain cerebrum myeloperoxidase activity in $CdCl_2$-induced neurotoxicity in Wistar rats. The data is represented as mean ± SD (n = 5). #Significant at $p < 0.05$ compared to the control; *Significant at $p < 0.05$ compared to $CdCl_2$ alone and $CdCl_2$ + gallic acid.

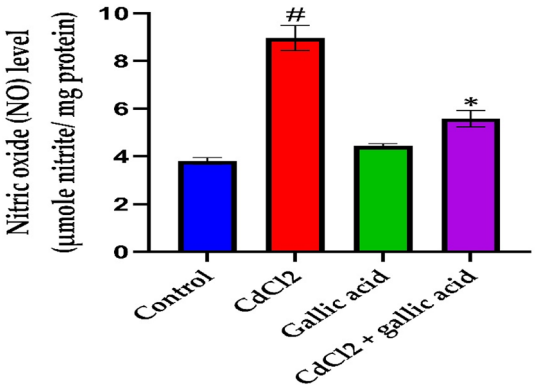

Figure 6. Impact of gallic acid on brain NO level in $CdCl_2$-induced neurotoxicity in Wistar rats. The data is represented as mean ± SD (n = 5). #Significant at $p < 0.05$ compared to the control; *Significant at $p < 0.05$ compared to $CdCl_2$ alone group.

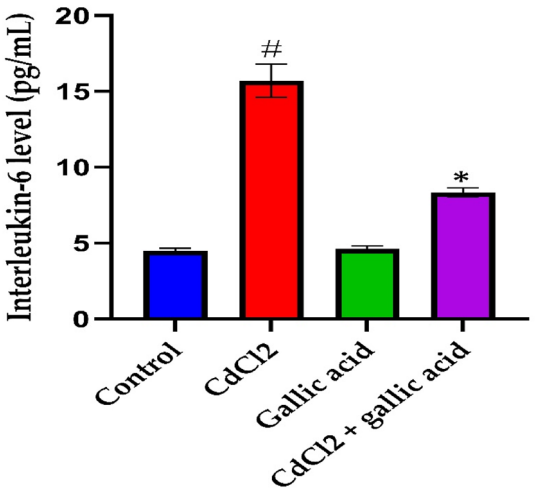

Figure 7. Impact of gallic acid on brain IL-6 level in $CdCl_2$-induced neurotoxicity in Wistar rats. The data is represented as mean ± SD (n = 5). #Significant at $p < 0.05$ compared to the control; *Significant at $p < 0.05$ compared to $CdCl_2$ alone group.

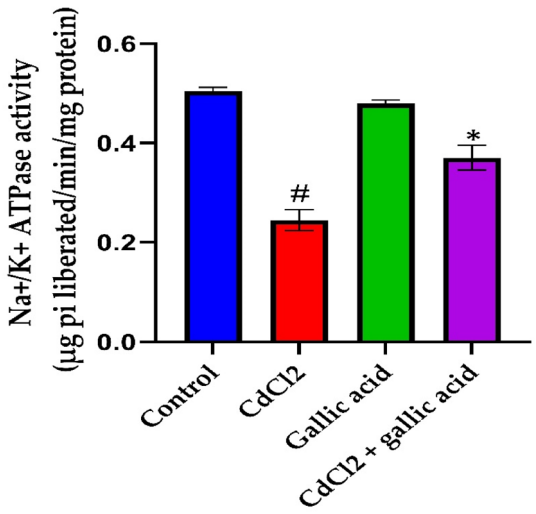

Figure 8. Impact of gallic acid and cadmium on the activities of membrane bound ATPases in the cerebrum of control and experimental rats. The data is represented as mean ± SD (n = 5). #Significant at $p < 0.05$ compared to the control; *Significant at $p < 0.05$ compared to the $CdCl_2$ only group.

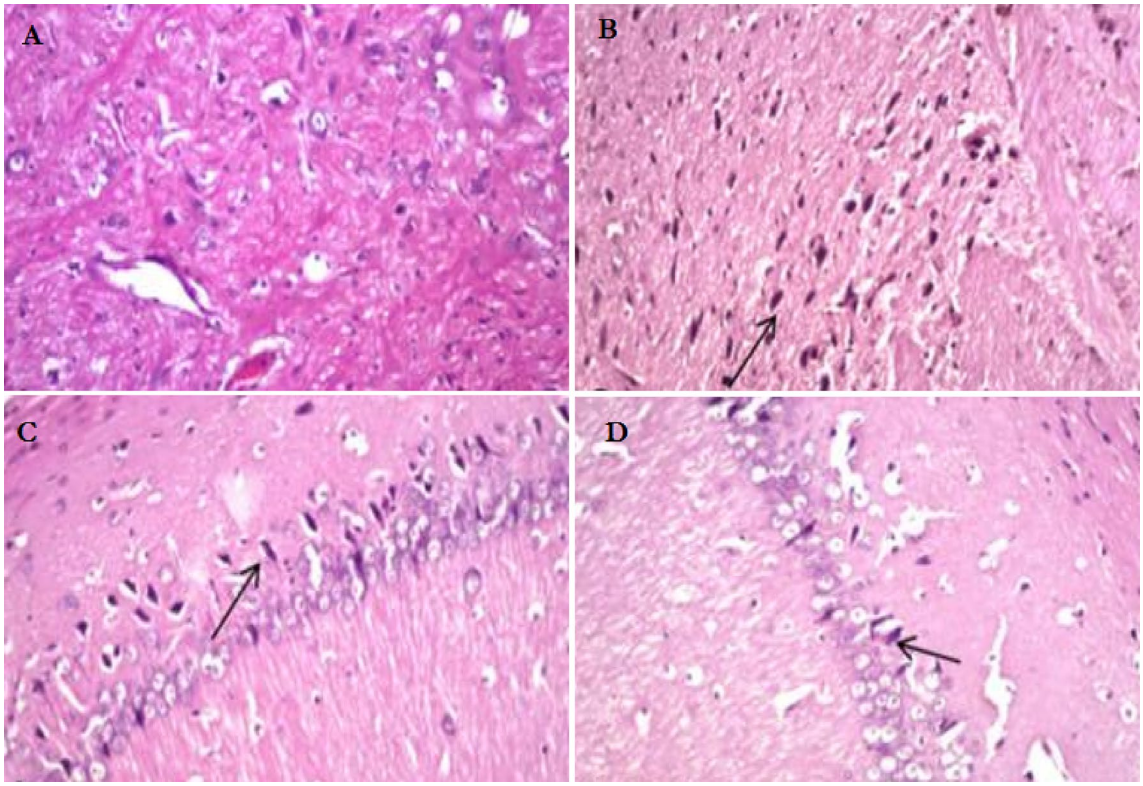

Figure 9. Impact of gallic acid on the cerebrum of $CdCl_2$-induced neurotoxic rats (H & E, 200X). Group A (Control rats), Group B (cadmium chloride-treated rats), Group C ($CdCl_2$ + GA-treated rats), Group D (gallic acid-treated rats) (**A**) Control showing normal granular layer, molecular layer, purkinje cell layer, blood vessels as well as while matter. (**B**) $CdCl_2$ group showing severe vacuolation of purkinje cell layer, optical empty spaces due to necrosis (arrow), severe separation of purkinje cell layer from granular layer, severe hemorrhage of white matter; (**C**) $CdCl_2$ + GA group showing vacuolated purkinje cell layer (black arrow), mild separation of purkinje cell layer; (**D**) GA group reveals normal granular layer, molecular layer, vacuolated purkinje cell layer (black arrow).

Shukla and Chandra[30] and Ojo et al.[13,34] found a similar decrease in catalase and SOD activities in rat brains after $CdCl_2$ intoxication. The restoration of SOD and catalase activities in the brains of rats suggests that gallic acid has protective potential by reducing lipid peroxidation in brain tissues. Meanwhile, a drop in GSH levels has been noted in some earlier studies. Due to the excessive availability of the lipid peroxidation product (MDA) and inhibition of enzymes by $CdCl_2$ metabolites, the level of GSH was lower in the current study. This could be due to its ability to conjugate electrophilic metabolites of $CdCl_2$. Gallic acid, on the other hand, may help in the management of ROS in the brain as a neuroprotective agent due to its ability to prevent $CdCl_2$-induced inhibition of GSH in the brain. The buildup of Cd in brain tissue, which depletes the GSH pool, may be the cause of the decrease in antioxidant enzyme levels observed in this study[35]. In addition to the binding of Cd to the sulfhydryl (-SH) group of the oxidative enzymes and inhibiting them, GSH depletion also renders the antioxidant enzymes inactive[36]. The current findings are consistent with those of Elkhadrag et al. and Ichipi Ifukor et al.[37,38]. Rutin, a well-known antioxidant, has been shown to have a similar effect on these antioxidant parameters[39].

The neurotoxin potency of Cd has been demonstrated in both in vitro and in vivo studies[40,41]. Animals exposed to Cd have been found to have altered catecholaminergic and serotoninergic transmission[42,43]. To determine the neurotoxic effects of heavy metals, it is imperative to investigate the activities of brain enzymes like AChE and BChE. According to several studies[44], AChE activity in the brain is believed to decline as a result of free radical production in the brain. Acetylcholine accumulation, which results in cholinergic hyperactivity, convulsions, and epileptic status, has been shown to be caused by decreased AChE[45]. Although it is unclear how much it directly contributes, decreased levels of AChE in the brain could be one of the indicators of Cd-induced injury because they are a key regulator of behavioral processes. The fact that $CdCl_2$ inhibited the activities of butyrylcholinesterase and acetylcholinesterase in the brains of rats in this study indicates that acetylcholine had accumulated due to disruptions cholinergic transmission. This result is consistent with Pari and Murugavel's report on the effect of diallyl tetrasulfide, which is commonly found in garlic, on AChE[46].

The most common neurotransmitter in the central nervous system, serotonin (5-HT), is essential for memory and learning[47]. On the other hand, tryptophan hydroxylase is distributed similarly to serotonin (5-HT) in the hypothalamus, the midbrain, and the limbic system of the brain[48–50]. In this study, rats treated with cadmium alone had considerably lower levels of 5-HT in their brains. By inhibiting the oxidative metabolism of serotonergic neurons, cadmium prevents tryptophan hydroxylase activity, which is essential for the conversion of tryptophan to 5-HT[51]. Our results are in line with those of Das et al.[52], who discovered that rats given $CdCl_2$ had markedly lower levels of 5-HT in their brains. In the current study, brain catecholamine levels (dopamine) increased in rats administered cadmium. It has been proposed that it causes changes in neurochemistry as a result of oxidative damage to brain tissue, which may be responsible for its toxic action[53,54]. Cd also promotes lipid peroxidation by increasing the production of superoxide anions[55], which act as primary oxidant. This process can cause direct cell damage by destroying membranes and indirect cell damage by producing reactive carbonyl products[56]. Cd-induced lipid peroxidation is the cause of membrane damage that leads to neuronal dysfunction, resulting in decreased dopamine uptake in the brain[57]. Our findings are consistent with[30], who found that low-dose chronic cadmium treatment markedly increased dopamine levels in rats. Additionally[58], found that low-dose chronic cadmium treatment elevated dopamine levels in rats.

Due to the presence of free fatty acids in the brain, it is extremely prone to lipid peroxidation[59]. The increase in MDA levels caused by $CdCl_2$ in the current study suggests that the brains of rats may experience cellular damage and dysfunction as a result of excessive ROS generation[60]. This supports the hypothesis that a decrease in glutathione levels is the first sign of oxidative stress. Rotimi et al.[14] made a similar finding of elevated MDA levels in $CdCl_2$-induced rats. On the other hand, gallic acid's capacity to restore MDA levels that are comparable to those of the relevant controls may be connected to its anti-peroxidative properties.

One of the defenders against neurotoxic assaults on a host's nervous system is neuroinflammation. As a promising clinical target, neuroinflammation is also a frequent pathological outcome or etiology of neurodegeneration and neurological disorders[61]. Therefore, the detection of specific neuroinflammation markers was crucial in this study. For example, the pathogenesis of inflammatory disorders and the maintenance of neural tissue homeostasis depend on the cytokine interleukin-6 (IL-6)[62]. Nitric oxide enhances cerebral blood flow while acting as a retrogressive neurotransmitter in synapses and is crucial for intracellular signaling in neurons[63]. Myeloperoxidase is a therapeutic target for oxidative stress and neuroinflammation-causing pathological processes[64]. The rise in IL-6 levels in the brains of rats suggests that it interferes with the physiological processes associated with neuroinflammation. This justify the role of IL-6 in the pathogenesis of neurodegenerative diseases. In this study, nitric oxide levels were higher than normal in the brains of rats due to $CdCl_2$, which may also have interfered with intracellular signaling in the brain. Reduced antioxidant status with elevated oxidative stress was associated with increased MPO activity in the rat brain. Gallic acid, on the other hand, has the potential to be an anti-inflammatory agent in inflammation-induced brain diseases, as evidenced by its ability to reverse the increase in NO and MPO induced by $CdCl_2$.

The production of metabolic energy, the uptake and release of catecholamines and serotonin, as well as neural excitability, are all regulated by the enzyme Na^+/K^+-ATPase[65,66]. Furthermore, Mg^{2+} -ATPase is responsible for keeping intracellular Mg^{2+} levels in the brain at a high level, where changes can affect the rate of protein synthesis and cell growth[67]. In the nervous system, Ca^{2+} acts as a second messenger. Numerous pathological lesions in the brain are caused by variations in Ca^{2+} levels[68] . The Ca2 + ATPase activity in the cell membrane is also inhibited by the Ca^{2+} overload caused by Cd, which ultimately results in irreversible cell death. Exposure to Cd affects the activities of these ATPase enzymes[17,43,69,70], indicating changes in membrane and neurotransmitter functions. Compared to control rats, $CdCl_2$ alone showed a significant decrease in ATPase enzyme activity in the brains of experimental animals. ATPase activity can be reduced due to the formation of Cd-ATPase complexes through the enzyme's SH group and/or increased oxidative stress[46]. Co-administration of gallic acid with Cd significantly increased the activity of ATPase enzymes in the brain compared to cadmium alone.

Significant histopathological changes in brain tissue have been widely associated with heavy metals[71]. According to histological examination of brain tissue, Cd intoxication causes abnormal structural changes in brain tissue, including apoptosis, nuclear vacuolization, and inflammatory lymphocytic changes. Cd alters the cortical micro-and macrostructures once it enters the brain. Cd destroys glial and neuronal cells in the hippocampus's white matter[72]. Cadmium causes severe damage to the brain, including encephalopathy and hemorrhage. These changes are neuropathological and neurochemical. The cortical neuroglia, pyramidal, and granule cells are also altered by Cd exposure[73]. Additionally, Cd affects the structure of nerve cells and parenchyma, impairing attention, memory, and olfactory functions as well as hypernociception[74]. According to a histopathological assessment of the cerebrum, the intoxication with cadmium led to abnormal ultra-structural changes, including severe vacuolation of purkinje cell layer, optical empty spaces due to cell necrosis, severe separation of purkinje cell layer from granular layer, severe hemorrhage, and cell degeneration. The observed pathological damage caused by $CdCl_2$-induced rats treated with gallic acid showed a significant recovery, indicating that gallic acid is capable of abating the neuronal impairment caused by cadmium. As a result, it might be hypothesized that gallic acid could prevent brain damage caused by Cd.

Our obtained data showed that $CdCl_2$ induced oxidative stress and neuroinflammation in the brains of Wistar rats. However, gallic acid offered neuroprotective effects on $CdCl_2$-induced toxicity in the brain, indicating its antioxidant and therapeutic potentials against oxidative stress and neuro-inflammation in the brain of rats exposed to $CdCl_2$. Our results suggested that gallic acid showed a profound improvement in neurotransmitter levels.

Materials and methods

Chemicals. Gallic acid (GA) was a product of SantaCruz Biotechnology, Heidelberg, Germany. ELISA kits for serotonin, dopamine, and interleukin-6 were purchased from Elabscience, Houston, Texas, USA. All other chemicals were of analytical grade.

Animals. For this experiment, twenty (20) Wistar rats weighing between 170 and 200 g were used. The animals were acquired from Landmark University's Biochemistry Department in Kwara State, Nigeria. Throughout the 21-day acclimatization period and the experimental period, they were kept at room temperature with unlimited access to food and water (21 days).

Ethical approval. All experimental rats used in this study were handled in accordance with the rules and regulations established for animal management in research, as outlined in NIH Publications No. 80-23 revised, 1996. The Animal Care and Use Ethical Committee at Landmark University, Nigeria (LUAC/2020/0052B) confirmed and approved that the experimental treatment of the rats is in accordance with ARRIVE guidelines. Furthermore, all methods/experimental protocol/s were approved by the institution's ethical committee and ARRIVE guidelines.

Animal groupings and experimental procedures. The rats were divided into four groups (n = 5) at random. The negative control group was given distilled water only for the duration of the study. Group 2 was the positive control, receiving only $CdCl_2$ (5 mg/kg/day) throughout the study. Group 3 received only 20 mg/kg of gallic acid per day, whereas Group 4 received 20 mg/kg of gallic acid + 5 mg/kg of $CdCl_2$. The doses of cadmium and gallic acid were chosen based on previous studies[6,75] for cadmium and[13,76] for gallic acid. Gallic acid was said to be safe at a daily dose of 20 mg/kg body weight[76], but it has been demonstrated that the cadmium dose chosen causes significant oxidative stress in various tissues[34,77].

Serum and organ collection. Animals were sacrificed under halothane anesthesia by cervical dislocation twenty-four hours after the last administration. Cardiovascular puncture was used to quickly collect blood into a plain bottle, which was then spun at 3000 rpm for 10 min to obtain serum, which was used to assess biochemical indices. Quickly removed from the body, the brain was rinsed in ice-cold normal saline and the cerebrum was homogenized in 0.1 M phosphate buffer (1:10 w/v) (pH 7.4). The brains were sectioned and the cerebrum were separated. At 3000 rpm, the homogenates were centrifuged. The obtained clear supernatant was used for various biochemical assays.

Biochemical analyses. Using Ellman's method[78], the activities of acetylcholinesterase (AChE) and butyrylcholinesterase were assessed. The TBA Reactive Substances (TBARS) concentration was measured using the Buege and Aust method[79]. The Na+/K+ ATPase's activity was assessed by Bonting[80]. The technique outlined by Misra and Fridovich[81] was used to test the superoxide dismutase activity. The catalase activity was tested using the procedure outlined by Aebi[82]. The method outlined by Ellman[83] was used to measure the level of reduced glutathione (GSH). The assay for myeloperoxidase activity was carried out using the technique described by Granell et al.[84]. The Griess reaction was used to calculate the nitric oxide level[85]. The levels of IL-6, dopamine, and serotonin were estimated using rat specific enzyme linked immunosorbent assay (ELISA) kits (ElabScience, USA) according to the manufacturer's instructions.

Histopathological investigation. Animal brain tissues were used for histopathological research. The tissues underwent routine processing before being formalin-fixed in 10% formalin and embedded in paraffin wax. Hematoxylin and eosin (H and E) were used to stain the cerebrum sections, which were of 4 μm thick, on glass

slides. A light microscope was used to examine the slides, and magnified images (200X) of the tissue structures were recorded[86].

Data analysis. Data analysis was performed using one-way analysis of variance (ANOVA) on GraphPad Prism 9.0, version (GraphPad software, Inc, San Diego, USA). All results were expressed as mean ± standard deviation (SD). Statistical significance at $p < 0.05$ was determined by using Tukey's post hoc multiple comparisons test.

Data availability

Data is available on reasonable request from the corresponding author.

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Author contributions

O.A.O., D.E.R., and A.B.O. conceptualized and designed the research study, D.E.R. and O.A.O. performed the study. O.A.O., D.E.R., A.B.O., A.D.O., and B.O.A. generated and curated the data. D.E.R., A.D.O., A.B.O., and O.A.O. wrote the first draft of the manuscript; O.A.O., A.B.O., A.D.O., and B.O.A. revised the manuscript for intellectual content. All authors approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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