Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 19-30 | ISSN:2251-6727

Acute Co-Exposure of Lead and Cadmium Induce Pronounced Neurotoxicity in Wistar Rat

Gayatri Sharma, Mitanshi Singh, Ashish Tiwari, S. K. Bunkar, P.J. John*

Department of Zoology, University of Rajasthan, Jaipur-302004.

***Corresponding author**: The IIS (deemed to be) University, Jaipur-302020.

(Received: 16 March 2025 Revised: 20 April 2025 Accepted: 01 May2025)

KEYWORDS

Heavy metals, acute toxicity, oxidative damage, neurotoxicity.

ABSTRACT: Heavy metals are known to disrupt central nervous system (CNS) function; their precise mechanisms of toxicity remain unclear. This study examines the acute neurotoxic effects of lead (Pb) and cadmium (Cd) on the cerebral cortex, hippocampus, and hypothalamus of Wistar rats (*Rattus norvegicus*). To investigate their impact, rats were divided into four groups: Group-I (Control), Group-II (administered lead acetate at 180 mg/kg b. w.), Group-III (given cadmium chloride at 26.4 mg/kg b. w.), and Group-IV (administered a combination of lead acetate at 180 mg/kg and cadmium chloride at 26.4 mg/kg b. w.). The designated treatments were given over a four-day acute period. The results revealed significant reductions in tissue protein levels, along with catalase (CAT) and superoxide dismutase (SOD) activities, across all respective brain areas. Additionally, lipid peroxide levels were significantly elevated, indicating oxidative stress. Glutathione peroxidase activity also showed a slight decrease. Among the groups, the combined exposure to Pb and Cd caused the more pronounced effects, with the hippocampus and hypothalamus being the most affected regions. Histopathological analysis further confirmed damage in all brain sub-regions examined. This study highlights that Pb and Cd can traverse the blood-brain-barrier (BBB), triggering oxidative stress and histopathological damage, thereby adversely affecting brain tissue. The findings emphasize the heightened neurotoxic effects when these metals are present in combination.

1. Introduction

Heavy metals are substances characterized by an atomic value exceed 20, a density exceeding 5 gram/cm³, and distinct metallic properties (1). Their increasing presence in the environment has become a significant concern for nearly all living organisms. The widespread use of chemical compounds to support industrial and technological advancements poses serious health risks in both developed and developing nations. While heavy metals have been naturally present in the Earth's crust since its formation, their extensive use has led to elevated concentrations in both terrestrial and aquatic ecosystems (2).

The World Health Organization has listed lead (Pb) as one of the compounds that is most harmful to human health (3). Lead (Pb) is a cumulative toxin that affects several bodily systems, such as the digestive, cardiovascular, neurological, and renal systems (4). The primary sources of lead exposure for the general population are food and water. Additionally, various occupational and recreational activities contribute significantly to lead exposure. These include handling firearms, brazing, painting, manufacturing explosives, glass glazing, jewellery-making, ceramics production, using lead-based water pipes, working with batteries, radiation shielding, and crafting etc. (5). Because lead is one of the minerals that plants absorb the most through their roots, its concentration levels are greater in agricultural products. The neurological system seems to be the primary and most vulnerable target of lead poisoning when contrasted to other organ systems (6). Lead (Pb) can traverse the blood brain barrier (BBB), where it disrupts normal neurological function by displacing essential zinc and calcium ions, leading to impairments in brain function. Pb also has the ability to induce apoptosis by expanding of free radicals, oxidative burden, and lipid per-oxidation can be brought on by imbalances resulting from modifications in

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 19-30 | ISSN:2251-6727

antioxidant homeostasis brought on by lead intoxication (7).

Another heavy metal that has drawn a lot of attention from the environmental and occupational sectors is cadmium (Cd). The main reason is that cadmium has an extended biological half-life and its slow excretion from the body. Through the zinc and calcium transporters, cadmium enters the nervous system and modifies the metal ions' homeostasis. Once inside the brain system, cadmium enhances the yield of reactive oxygen species (ROS) while reducing ATP synthesis, thereby disrupting mitochondrial respiration (8). Cd accumulates over time in a number of tissues, including the kidney, liver, central nervous system (CNS), and peripheral neural system (PNS) (9). By inducing perturbations to neurotransmitter signalling proteins and increasing asynchronicity in neurotransmitter release, cadmium also affects normal neurotransmission. Furthermore, cadmium affects the control of glycogen metabolism and the blood–brain barrier. These processes collectively represent several areas of biochemical disruption leading to cumulative damage to the nervous system, potentially raising the risk of neurological and neurodegenerative diseases (10).

Exposure to chemical mixtures through ingestion and inhalation is a significant factor influencing toxicity. However, few in vivo studies have assessed the combined effects of chemical mixtures versus single substances. A study investigating workplace exposure to cadmium (Cd) and lead (Pb) among workers in a nonferrous metal smelter found that the combined exposure influenced oxidative stress markers in the blood. The observed changes involved variations in malondialdehyde, glutathione, glutathione-S-transferase, and 8-Hydroxy-2-deoxyguanosine (8-OHdG) concentrations (11). Concerns persist regarding the potential synergistic toxicity of Pb and Cd due to their shared target organs and mechanisms of action. However, research on how co-exposure to these metals impacts the central nervous system (CNS) in animal models remains limited. The purpose of this study is to evaluate the acute neurotoxic effects of Pb and Cd, together individually and synergistically, in Wistar rats.

2. Methods

Chemicals: Lead acetate (Product No. 72518, CAS No. 6080-56-4) and cadmium chloride (Product No. 99643, CAS No. 10108-64-2, ASC 99%) were sourced from Sisco Research Laboratories Private Limited, Maharashtra, India. Another laboratory chemicals used in the study were acquired from Sigma-Aldrich USA, HiMedia Mumbai, India, Merck Germany, and Rankem Thane, India. Ultrapure water, crucial for preparing reagents and buffers in biochemical assays, was provided by a Millipore Water sanitation System which was Direct-Q® Water Purification System from Merck Millipore and utilized consistently during the experimentation.

Test animals: Male Wistar rats (*Rattus norvegicus*), 3 months old and measuring 250–280 g, were obtained from the National Institute of Biologicals (NIB), Noida-201 309 (U.P.), India. The rats were maintained in a well-structured environment, Aerated animal facility with a regular day-night cycle, and the temperature was regulated between 23°C and 25°C. They were housed in polypropylene enclosure with wood shaving bedding. Food (Hindustan Lever Limited, New Delhi, India) and water were available to them at all times. The Institutional Animal Ethics Committee (IAEC) approved this study (CCSEA proposal no. UDZ/IAEC/2023-I/10), which was directed in accordance with the outlines established by the Committee for Control and Supervision of Experiments on Animals (CCSEA), under the Government of India, New Delhi. Prior to the experiment, the rats underwent a 15-day acclimatization period. They were then randomly assigned to four groups, with each group consisting of five rats. Group I acted as the control and received distilled water daily. Groups II, III, and IV were designated as experimental groups. Group II was given lead acetate at a dose of 180 mg/kg b. w., while Group III received cadmium chloride at 26.4 mg/kg b. w.—both representing 30% of the reported LD50. Group IV was exposed to a combination of lead acetate (180 mg/kg b. w.) and cadmium chloride (26.4 mg/kg b. w.). All treatments were administered orally over four consecutive days.

The animals were closely monitored for signs of toxicity, as well as their food and water intake. Following the final administration, they were euthanized under mild anesthesia and subjected to autopsy. The brain tissues were carefully extracted, and the neurosomatic index (NSI) was calculated for each group of animals. Following extraction, the brains were rinsed with chilled saline solution and separated into

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 19-30 | ISSN:2251-6727

distinct regions, such as the cerebral cortex, hippocampus, and hypothalamus. These tissue samples were prepared for neurochemical analysis and stored at -20°C until further examination. Additionally, portions of the tissues were fixed in a preservative solution for histopathological evaluation.

The entire protein composition in neural tissue was assessed using the Bradford Assay, with bovine serum albumin (BSA) as the mention standard (12). Lipid peroxidation, measured as malondialdehyde (MDA) formation, served as an indicator of TBARS (thiobarbituric acid reactive substances) and was assessed following the method outlined by Ohkawa et al. (13). Tissue GPx (glutathione peroxidase) enzymatic activity was analyzed as per the procedure described by Wood (14). Superoxide dismutase (SOD) function in neural tissue was assessed using the technique developed by Marklund and Marklund (15). Catalase (CAT) enzyme activity was measured according to the method outlined by Aebi (16).

The changes in the expression of antioxidant genes due to the lethality of lead (Pb) and cadmium (Cd) in the specific brain regions, such as the cerebral cortex, hypothalamus, and hippocampus were done by RT-PCR analysis.

For RNA extraction, frozen brain tissues were thawed and homogenized. Complete RNA was extracted using the TRIzol method, followed by Verso cDNA Synthesis Kit were used for synthesis of complementary DNA (cDNA) by following the manufacturer's instructions. The RNA amount and purity were evaluated using a Nanodrop spectrophotometer (VWR mySPEC) ensuring an A260/A280 ratio of at least 2.0 to confirm suitability for further analysis.

3. To analyze gene expression, primers specific to SOD, GPX, and catalase, along with the housekeeping gene β-actin, were used in the quantitative PCR (qPCR) reaction. PowerUp SYBR Green PCR premix was used for the amplification of cDNA samples on an Applied Biosystems 7500 Fast Real-Time PCR system. The qPCR was performed following the manufacturer's recommended amplification conditions. To ensure specificity, PCR products were analyzed using agarose gel electrophoresis, and melting curve analysis was performed. Negative controls were included to rule out contamination or non-specific amplification.

The gene expression data were processed using the $2^{-\Delta\Delta CT}$ method, where expression levels of the target genes (GPX, SOD, and catalase) were normalized to β-actin. This allowed for the comparison of gene expression between the control and metal-exposed groups, as well as between the different brain regions. The results provide insights into how Pb and Cd exposure may alter the oxidative stress response in key brain areas.

Table 1: The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assay uses the forward and reverse sequences of primers to assess the expression of linked genes.

Genes name	Sequences of Primers (5'-3')	Genes size	Sequence ID or Accession no.
Catalase	F-GTGCATGCATGACAACCAGG R-GAATGTCCGCACCTGAGTGA	163 bp	NM_012520.2
SOD1	F-AATGTGTCCATTGAAGATCGTGTGA R-GCTTCCAGCATTTCCAGTCTTTGTA	141 bp	NM_017050.1
SOD2	F-AAGGGAGATGTTACAACTCAGG R-GCTCAGGTTTGTCCAGAAAATG	96 bp	NM_017051.2
GPx	F-CCAGCTACTGAGGTCTGACAG R-ACTTGAGACTAGGCAGGATCTC	153 bp	NM_022525.4

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 19-30 | ISSN:2251-6727

Histopathological assessment: Brain tissues from both the control and experimental groups were preserved in ten percent buffered formalin for 24 hours. The tissues were progressively dehydrated with ascending concentrations of alcohol (70%, 90%, and 100%) for 30 minutes at each stage, followed by a 10-minute clearing process in xylene. Subsequently, the tissues were immersed in a 2:10 mixture of xylene-alcohol for two hours before being embedded in paraffin at 60°C and left overnight.

The next day, tissue blocks were engrained in paraffin wax, and 5 µm thick slices were cut using a microtome. All slices were cut in the coronal plane to facilitate histological analysis of the cerebral cortex, hypothalamus, and hippocampus. The slides were dewaxed by using xylene, rehydrated through a graded series of ethanol solutions, and subsequently stained with hematoxylin and eosin. The processed sections were then examined under a Leica DM 1000 compound light microscope (Wetzlar, Germany), and images were captured using a DFC 450C digital camera.

4. **Statistical Assessment:** Statistical analysis was done by using the one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Data are depicted as Mean (± SEM). Different superscripts (***,**,*) within the same row indicate extremely significant ($P < 0.001$),highly significant ($P < 0.01$), and significant ($P < 0.05$) differences, respectively, in contrasted with the control group.

5. Results

Impact of Pb and Cd on Body Weight, Neurosomatic Index, and Protein Concentration

Exposure to cadmium (Cd) and lead (Pb), either individually or in combination, for four days did not result in significant changes in the neurosomatic index. Whereas, a slight, insignificant reduction in average body weight was noticed in the treated groups (Fig. 1A), indicating that the brain mass-to-body mass ratio remained largely unaffected by metal exposure. In contrast, a notable reduction in tissue protein concentrations was recorded across all neuronal sub-regions in the Pb- and Cd-exposed groups compared to the control (Fig. 1B). The most substantial decline was observed in the group administered to both Pb and Cd, suggesting a possible synergistic toxic effect.

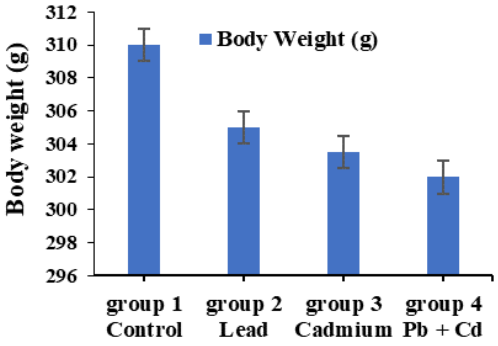


Fig.1A

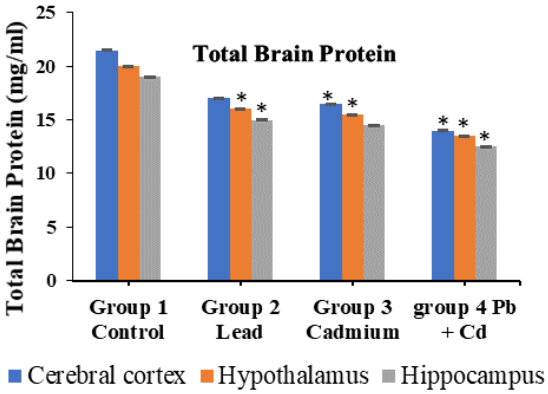


Fig.1B

Fig. 1: The impact of acute Pb and Cd administration on body weight (Fig. 1A) and total tissue protein in brain sub-regions (Fig. 1B) is presented. Values represent Mean ± SEM bearing different superscripts in the same rows differs significantly ($P<0.05$ from Control group).The sample size (N) for each group was as follows: control (5), 180 mg/kg b. w. Pb (5), 26.4 mg/kg b. w. Cd (5), and the combined 180 mg/kg b. w. Pb + 26.4 mg/kg b. w. Cd group (5).

Impact of Pb and Cd on Lipid Peroxidation

Oxidative stress resulting from lead and cadmium exposure was evident through increased lipid peroxidation across all brain sub-regions (Fig. 2). A significant rise in lipid peroxidation, marked by elevated malondialdehyde (MDA) levels, was observed in the hypothalamus ($P < 0.05$). In the cerebral cortex and hippocampus, the combined exposure led to a more pronounced effect, resulting in a statistically relevant elevation in lipid peroxidation ($P < 0.01$).

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 19-30 | ISSN:2251-6727

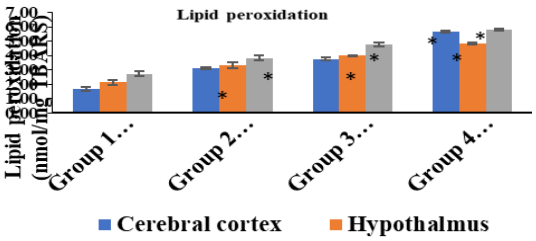


Fig. 2

Fig. 2. Lipid peroxidation (nmol TBARS formed/hour/mg protein) following acute exposure to Pb and Cd. Values represent Mean ± SEM bearing different superscripts in the same rows differs significantly (P<0.01 from Control group). The sample size (N) for each group was as follows: control (5), 180 mg/kg b. w. Pb (5), 26.4 mg/kg b. w. Cd (5), and the combined 180 mg/kg b. w. Pb + 26.4 mg/kg b. w. Cd group (5).

Effect on glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities and antioxidant gene expression levels: Exposure to 26.4 mg/kg b. w. of cadmium chloride and 180 mg/kg b. w. of lead acetate resulted in a dose-dependent decrease of glutathione peroxidase (GPx) in the treated groups, with the combination treatment exhibiting the most substantial effect (Fig. 3A). Furthermore, a statistically remarkable reduction in SOD enzyme activity was recorded in the cortex, hypothalamus, and hippocampus of treated groups contrasted to control animals ($P < 0.01$; Fig. 4A).Likewise, CAT function was significantly decreased in all brain sub-regions in the treated groups compared to controls ($p < 0.001$; Fig. 5A). The changes in the gene expression of antioxidant genes such as GPx, SOD and CAT showed the same patterns of the respective enzyme activity levels (Fig. 3B, 4B & 5B).

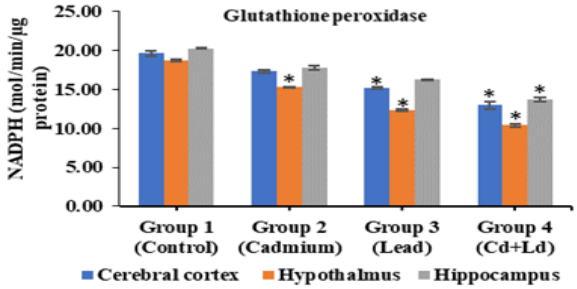


Fig. 3A

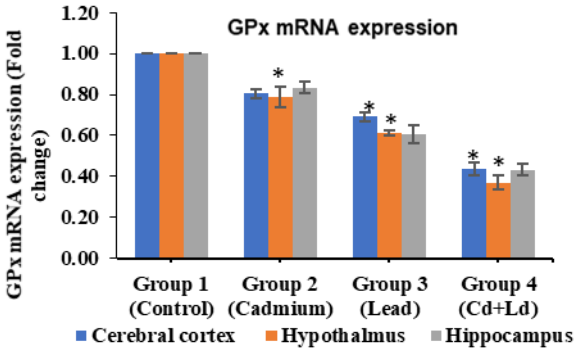


Fig.3B

Fig. 3.Glutathione peroxidase (GPx): nmol NADPH oxidized/min./mg protein and GPx-mRNA expression (fold change). Effect of Acute treatment to Pb and Cd on Glutathione Peroxidase (GPx) Activity (A) and GPx Gene Expression (B):The data are depicted as Mean (± SEM), bearing different superscripts in the same rows differs significantly (P<0.01 from Control group).. The sample size (N) for each group was as follows: control (5), 180 mg/kg b. w. Pb (5), 26.4 mg/kg b. w. Cd (5), and combined 180 mg/kg b. w. Pb + 26.4 mg/kg b. w. Cd (5).

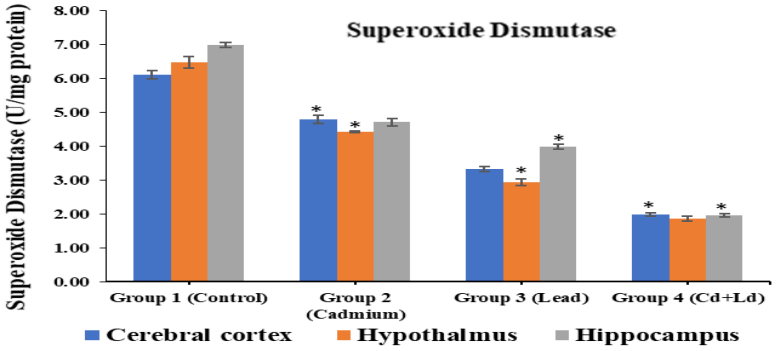


Fig. 4A

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 19-30 | ISSN:2251-6727

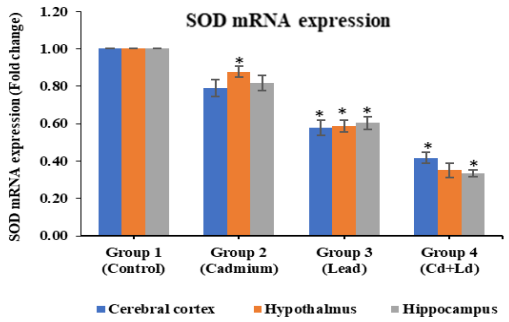


Fig. 4B

Fig. 4.Superoxide dismutase (SOD): (Specific activity of SOD (units/mg protein) and SOD-mRNA expression (fold change). Effect of Acute administration to Pb and Cd on Superoxide Dismutase (SOD) enzyme Activity (A) and SOD Gene Expression (B): The data are displayed as Mean (± SEM), with values marked by distinct superscripts (*) within the same row indicating a statistically relevant variations (P < 0.01) correlated with the control group. The sample size (N) for each group was as follows: control (5), 180 mg/kg b. w. Pb (5), 26.4mg/kg b. w. Cd (5), and combined 180 mg/kg b. w. Pb + 26.4.

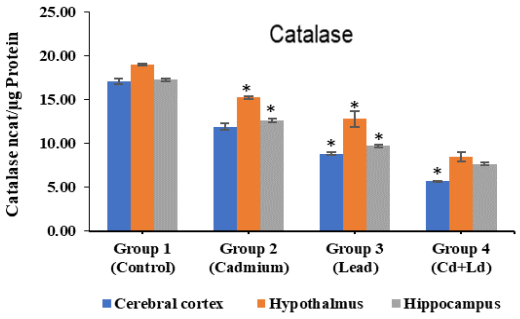


Fig. 5A

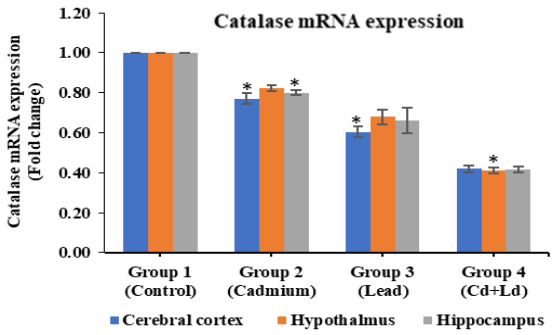


Fig. 5B

Fig. 5. Catalase (CAT): (Specific activity of CAT (units/mg protein) and CAT-mRNA expression (fold change). Effect of Acute administration of Pb and Cd on Catalase (CAT) Activity (A) and CAT Gene Expression (B):The results are expressed as Mean (± SEM), with values annotated with distinct superscripts (*) within the same row indicating a statistically relevant difference (P < 0.01) relative to the control group. The sample size (N) for each experimental group was as follows: control (5), 180 mg/kg b. w. Pb (5), 26.4 mg/kg b. w. Cd (5), and combined 180 mg/kg b. w. Pb + 26.4 mg/kg b. w. Cd (5).

Morphological measurement and Histopathological Alterations in H&E-Stained Slices of the Hippocampus and Cerebral Cortex: Photomicrographs of hematoxylin and eosin (H&E)-dyed coronal slices of the cerebral cortex and the hippocampus of control animals and treated animals are given in the fig. 6, 7, 8 & 9.

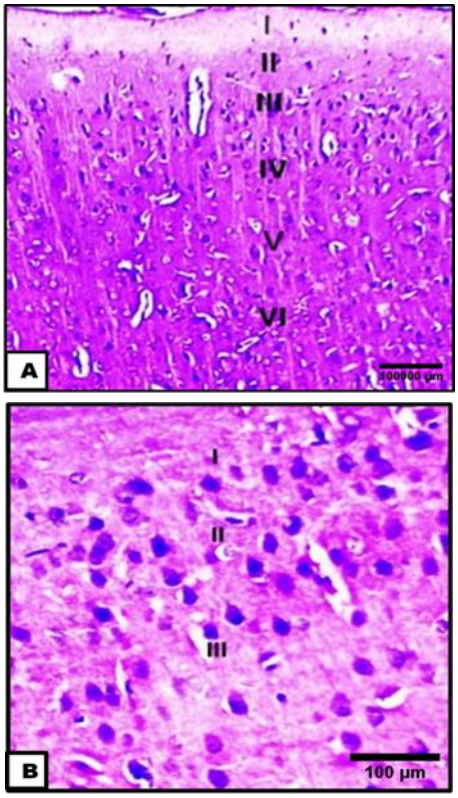


Fig. 6.The cerebral cortex of the control group exhibits six distinct layers: the first one is molecular layer, second is outer granular layer, third one is outer pyramidal layer, fourth inner granular layer, fifth inner pyramidal layer, last one is polymorphic layer shown(I),

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 19-30 | ISSN:2251-6727

(II),(III),(IV), (V), and (VI) respectively. Hematoxylin and eosin (H&E) staining dyes was used for visualization at (A) ×100 magnification and (B) ×400 magnification.

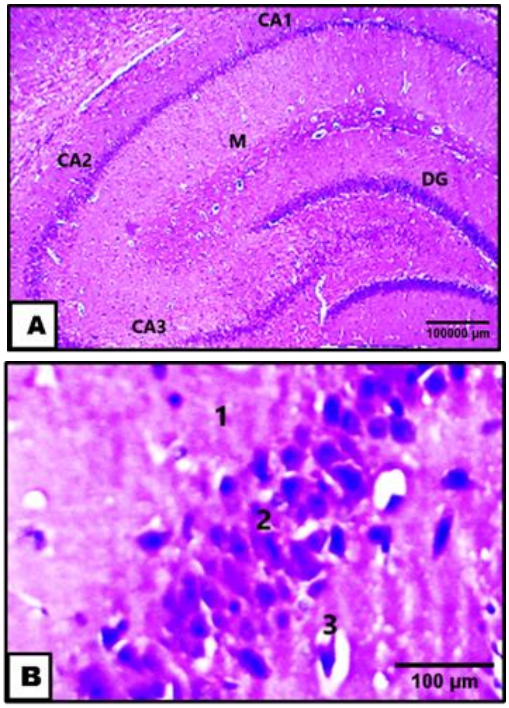
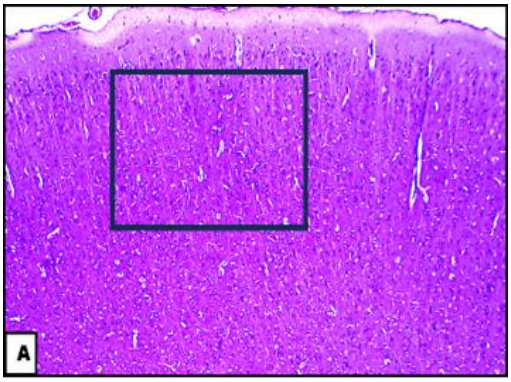


Fig. 7.(A) The hippocampus in the control brain includes distinct regions such as Cornu Ammonis (CA1, CA2, CA3) and the Dentate Gyrus (DG), with the molecular layer (M) situated within their concavity. (A) H&E staining at ×100 magnification. (B) The hippocampus exhibits its specific three layers: first is the molecular layer (1), second is pyramidal layer (2), and third one is polymorphic layer (3).



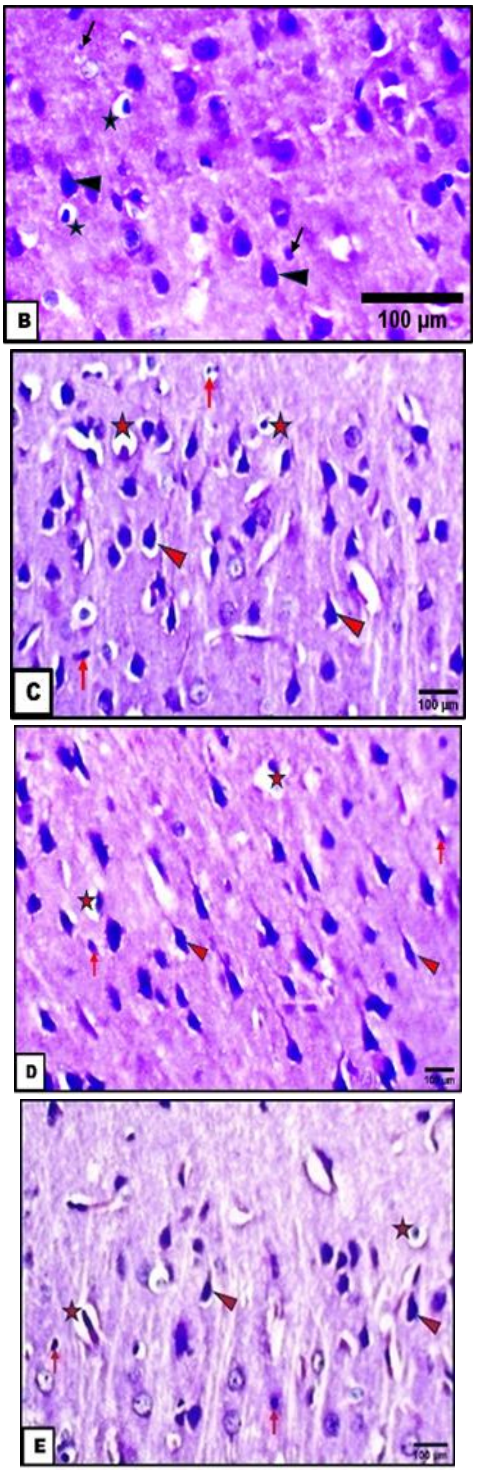
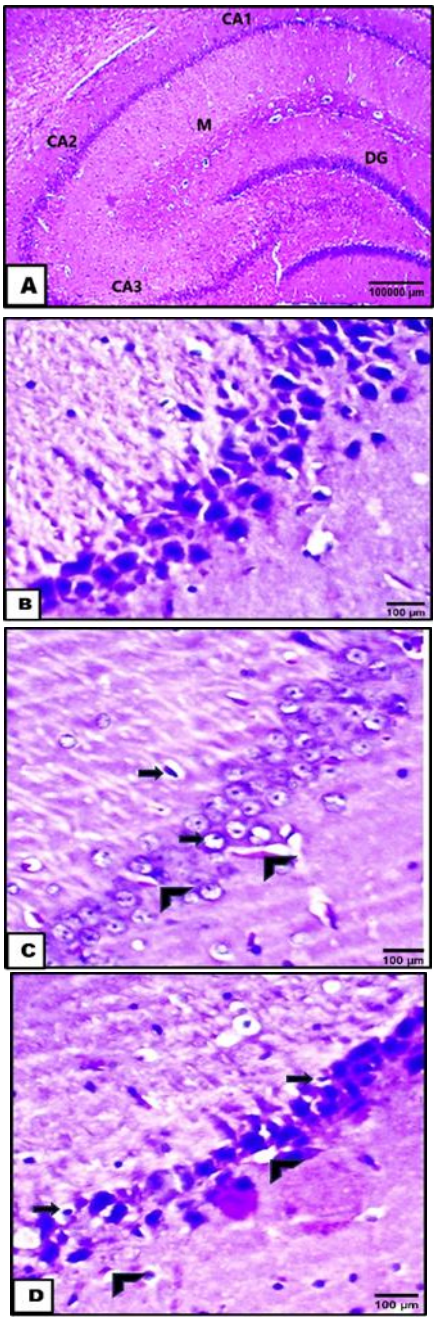


Fig. 8. Morphological assessment of the cortex: Histological analysis revealed normal cortical architecture in the control group, as observed in H&E-stained sections (B) at ×400 magnification. In contrast, Pb-treated (C), Cd-treated (D), and Pb + Cd-treated (E) groups exhibited neuronal degeneration, including

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 19-30 | ISSN:2251-6727

shrunken, hyperchromatic neurons, irregular Nissl body distribution (long red arrow), distorted neurons with intensely stained nuclei (red arrowhead), and vacuolization (red star). (n = 5 per group, *P < 0.05). Magnifications: A (H&E ×100), B–E (H&E ×400). Sample size per group: control (5), Pb (180 mg/kg b. w., 5), Cd (26.4 mg/kg b. w., 5), and Pb + Cd (5).



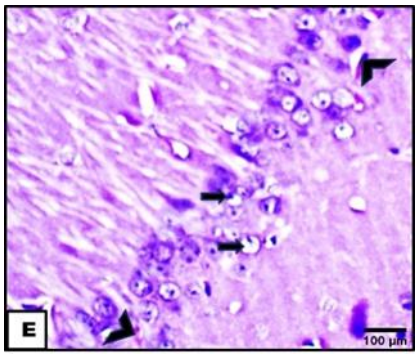


Fig. 9. Hippocampal morphological assessment: Morphological alterations in the hippocampus following Pb and Cd exposure were analyzed. (A) The hippocampus forms a C-shaped structure, including CA1, CA2, CA3, and the dentate gyrus (DG). The control group (B) displayed normal histological features in the CA3 region. In contrast, Pb (C), Cd (D), and Pb + Cd (E) treatments led to reduced pyramidal cell layer thickness, along with atrophic transformations such as degenerated, intensely stained neurons, disrupted chromatolytic process, disorganized Nissl substance distribution (arrowhead), and distended blood vessels (arrow). Magnifications: A (H&E ×100), B–E (H&E ×400). Scale bar = 100 µm. Sample size per group: control (5), Pb (180 mg/kg) (5), Cd (26.4 mg/kg) (5), and Pb + Cd (5).

Acute exposure to lead (Pb) and cadmium (Cd) results in significant structural and cellular alterations in the hippocampus and cortex of the brain, as evidenced by histopathological examination using Hematoxylin and Eosin (H&E) staining. In the control group, normal cellular architecture was observed, with the hippocampus displaying its characteristic C-shaped structure and The Cornu Ammonis (CA1, CA2, CA3) regions each contain distinct layers, including the polymorphic, pyramidal, and molecular layers. The polymorphic layer features neuronal processes, astroglia cells, and capillary vasculature, while the pyramidal layer consists of pyramidal neurons with vesicular nuclei and basophilic cytoplasm. This well-preserved structure reflects normal cellular organization and function.

6. Discussion

Heavy metals are frequently present in the environment. Agricultural and industrial practices increase the likelihood that they may enter the food chain.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 19-30 | ISSN:2251-6727

Eventually, heavy metals like Pb and Cd build up in the body of organisms and remain there for a long time; have a variety of negative impacts (17). The toxicity of metal mixtures is rarely studied, despite the fact that the reports on negative impacts of individual metals are available in plenty. Furthermore, there is limited knowledge regarding the neurotoxic effects of Pb and Cd metal combinations at low concentrations. Since the widespread presence of Pb and Cd in the environments and the lack of study of its co-exposure it is imperative to elucidate the neurotoxicological characteristics of amalgamated Pb and Cd in Wistar rat for acute period.

Andjelković et al. (18) investigated the toxicity due to acute administration to cadmium (Cd) and lead (Pb) in the kidney, blood and liver, of adult Wistar rats. Their findings highlighted redox imbalance as a key mechanism of toxicity, revealing significant disturbances in redox balance within the affected tissues. The study also demonstrated that metal mixtures induced more severe toxicity compared to individual metal exposure.

Similarly, Wang et al. (19) examined the impact of chronic administration to multiple heavy metals in rats, reporting varying degrees of metal accumulation across different tissues and organs. Additionally, Liu et al. (20) explored the combined exposure of Pb and arsenic (As) in zebra fish, observing substantial brain damage, behavioural impairments, and alterations in neurotransmitter levels, further emphasizing the heightened neurotoxic effects of metal mixtures.

Superoxide dismutase (SOD) is a crucial antioxidative catalyst responsible for catalyzing the conversion of superoxide radicals (O_2^-), into hydrogen peroxide (H_2O_2) and oxygen (21). The significant decrease in SOD enzyme functions observed in the brain sub-regions following exposure to cadmium and lead indicates that these metals disrupt the cellular antioxidant defence system. Cd and Pb are known to trigger reactive oxygen species (ROS) or interfere with metal cofactors required for SOD activity, leading to reduced enzyme function (22). This impairment may be due to direct binding of these metals to SOD or by altering the cellular redox state, thereby hindering the enzyme's ability to neutralize superoxide radicals (23). The reduction in CAT activity in the treated rats suggests that lead and cadmium exposure increase the burden of ROS in the brain, leading to an overproduction of H_2O_2 that overwhelms the antioxidant defence systems. The depreciation in CAT enzyme activity could be a direct result of metal-induced inhibition of the enzyme or due to increased oxidative damage to the enzyme itself, reducing its capacity to neutralize hydrogen peroxide (24).

GPx is essential for safeguarding cells against oxidative damage by neutralizing peroxides, including lipid hydroperoxides, using glutathione as a cofactor (25). The dose-dependent reduction in GPx levels observed in the treated animals suggests that subjection to Cd and Pb disrupts the normal functioning of this enzyme. Lead and cadmium may deplete glutathione stores or inhibit GPx enzyme activity through direct binding, thus impairing the ability to neutralize lipid peroxides and other harmful peroxides. The combination treatment exhibited a more pronounced effect, which may be due to synergistic toxicity, where both metals exacerbate oxidative stress and inhibit antioxidant enzyme activity more severely than when each metal is present alone.

Lipid peroxidation is a vital sign of oxidative damage, where reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide attack lipids in cellular membranes, resulting in the formation of malondialdehyde (MDA) and other reactive aldehydes (26,27). The remarkable increment in lipid peroxidation, particularly in the hypothalamus, cerebral cortex, and hippocampus, indicates that Cd and Pb exposure induces severe oxidative harm, leading to membrane damage and the formation of LPO products. This effect was more pronounced in the combination group, which suggests that exposure to both metals together have a synergistic effect, further increasing ROS production and lipid peroxidation.

Yalin et al. (28) reported a notable elevation in malondialdehyde (MDA) proportions, along with declined superoxide dismutase (SOD) and catalase (CAT) functions in kidney and liver of ovariectomized rats. These observations are continued with our results, demonstrating that cadmium induces oxidative stress by impairing antioxidant defences. Additionally, their study showed that ovariectomy exacerbated cadmium toxicity, implying a protective role of estrogens. Although we did not examine hormonal influences, their results suggest that sex differences may influence susceptibility to cadmium-induced neurotoxicity.

Similarly, the study by Poli et al., (29) demonstrated cadmium-induced oxidative damage in Wistar rats' liver and kidney tissues, showing diminished the

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 19-30 | ISSN:2251-6727

functionality of antioxidant enzymes like catalase (CAT), and superoxide dismutase (SOD) accompanied by increased lipid peroxidation (LPO).Our study extends this understanding to the brain, illustrating that combined exposure to lead and cadmium significantly reduces SOD, CAT, and glutathione peroxidase (GPx) activities while increasing LPO levels in brain tissues.

The brain is highly susceptible to oxidative deterioration due to its elevated metabolic activity, high oxygen exhaustion, and the presence of poly-unsaturated fatty acids in neuronal membranes, which are particularly prone to peroxidation (30). However, different brain regions have varying levels of antioxidant defences, and this could explain why some areas are more affected than others (31). The hippocampus, for example, is involved in learning and memory and has been shown to be particularly vulnerable to oxidative stress (32). The hypothalamus, involved in regulating homeostasis and neuroendocrine functions, is also sensitive to oxidative damage, which may explain the observed changes in lipid per-oxidation and antioxidant protein activity in this region (33).

Hegazy et al., (34), demonstrated that Pb exposure leads to cortical disorganization, vacuolation in the molecular layer, and widespread neuronal shrinkage. They reported the separation of the pia mater from the cortical surface, a hallmark of Pb-induced neurotoxicity. The presence of congested blood vessels and widened perivascular spaces in their study further supports the notion that Pb disrupts cerebrovascular integrity, increasing susceptibility to oxidative stress and apoptosis. The more pronounced histopathological effects in the combination group may be due to a synergistic interaction between Cd and Pb. Both metals can independently generate ROS, and when combined, their combined toxic effects may overwhelm the brain's antioxidant defences. The increase in lipid peroxidation in the cerebral cortex and hippocampus in the combination group suggests that these regions are particularly vulnerable to the synergistic oxidative damage caused by both metals.

In the hippocampus, Pb and Cd exposure resulted in reduced pyramidal cell layer thickness and significant dystrophic changes. Treated neurons appeared shrunken and intensely stained nuclei, with aberrant chromatolysis, disorganized Nissl substance distribution, and vacuolization—hallmarks of neuronal damage. These alterations suggest potential cell death or functional impairment in protein synthesis and metabolism. Vacuolization and dilated blood vessels indicate possible blood-brain barrier disruption, further exacerbating neuronal injury. Such morphological changes align with established neurotoxicity mechanisms, synaptic plasticity, and cognitive and motor abilities (35). The findings suggest that acute exposure to Pb and Cd leads to neuronal degeneration in these regions which may potentially affect the cognitive processes such as memory, learning, and overall brain functions.

References

1. Ali, H., Khan, E., & Ilahi, I. (2019). Environmental chemistry and ecotoxicology ofhazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. InJournal of Chemistry (Vol. 2019). Hindawi Limited.https://doi.org/10.1155/2019/6730305.
2. Gautam, P. K., Gautam, R., Banerjee, S., Chattopadhyaya, M., & Pandey, J. (2016).Heavy metals in the environment: Fate, transport, toxicity and remediation technologies.In Heavy Metals: Sources, Toxicity and Remediation Techniques (pp. 101–130).
3. Awadalla, A., Mortada, W. I., Abol-Enein, H., & Shokeir, A. A. (2020). Correlation between blood levels of cadmium and lead and the expression of microRNA-21 inEgyptian bladder cancer patients. Heliyon, 6(12).https://doi.org/10.1016/j.heliyon.2020.e05642
4. Saleh, S., & Meligy, F. (2018). Study on Toxic Effects of Lead Acetate on CerebellarCortical Tissue of Adult Albino Rats and the Role of Vitamin E as a Protective Agent.Ain Shams Journal of Forensic Medicine and Clinical Toxicology, 31(2), 110–118.22https://doi.org/10.21608/ajfm.2018.15884
5. Levin, R., Brown, M. J., Kashtock, M. E., Jacobs, D. E., Whelan, E. A., Rodman, J.,Schock, M. R., Padilla, A., & Sinks, T. (2008). Lead Exposures in U.S. Children, 2008:Implications for Prevention. Environmental Health Perspectives, 116(10), 1285–1293.https://doi.org/10.1289/ehp.11241
6. Hegazy, A. A., Domouky, A. M., Akmal, F., & El-wafaey, D. I. (2023a). Possible roleofselenium in

ameliorating lead-induced neurotoxicity in the cerebrum of adult male rats:an experimental study. Scientific Reports, 13(1). https://doi.org/10.1038/s41598-023-2042319-3

7. Singh, P. K., Singh, M. K., Yadav, R. S., Nath, R., Mehrotra, A., Rawat, A., & Dixit, R.K. (2019). Omega-3 fatty acid attenuates oxidative stress in cerebral cortex, cerebellum,and hippocampus tissue and improves neurobehavioral activity in chronic lead-inducedneurotoxicity. Nutritional Neuroscience, 22(2), 83–97.https://doi.org/10.1080/1028415X.2017.1354542
8. Wang, B. & Du, Y. (2013). Cadmium and its neurotoxic effects. In OxidativeMedicine and Cellular Longevity. https://doi.org/10.1155/2013/898034
9. Arruebarrena, M. A., Hawe, C. T., Lee, Y. M., & Branco, R. C. (2023). Mechanisms ofCadmium Neurotoxicity. International Journal of Molecular Sciences, 24(23), 16558.19https://doi.org/10.3390/ijms242316558
10. Ma, Y., Su, Q., Yue, C., Zou, H., Zhu, J., Zhao, H., Song, R., & Liu, Z. (2022). TheEffect of Oxidative Stress-Induced Autophagy by Cadmium Exposure in Kidney, Liver,and Bone Damage, and Neurotoxicity. International Journal of Molecular Sciences,23(21), 13491. https://doi.org/10.3390/ijms232113491
11. Matović, V., Buha, A., Dukić-Ćosić, D., & Bulat, Z. (2015). Insight into the oxidativestress induced by lead and/or cadmium in blood, liver and kidneys. Food and ChemicalToxicology, 78, 130–140. https://doi.org/10.1016/J.FCT.2015.02.011
12. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgramquantities of protein utilizing the principle of protein-dye binding. AnalBiochem. 72:248-54. doi:10.1016/0003-2697(76)90527-3.21
13. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for Lipid Peroxides in Animal Tissuesby Thiobarbituric Acid Reaction. In Analytical Biochemistry (95).
14. Wood, J. L. (1970). Biochemistry of mercapturic acid formation. Metabolic Conjugationand Metabolic Hydrolysis, 261–299.https://doi.org/10.1016/B978-0-12-257602-7.50012-3
15. Marklund, S. & Marklund, G. (1974). Involvement of the Superoxide Anion Radical inthe Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase.European Journal of Biochemistry, 47(3), 469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x
16. Aebi, H. (1974) Catalase. In: Bergmeyer, H.U., Ed., Methods of Enzymatic Analysis,Verlag Chemie/Academic Press Inc., Weinheim/NewYork, 673-680.http://dx.doi.org/10.1016/b978-0-12-091302-2.50032-3
17. Rai, P. K., Lee, S. S., Zhang, M., Tsang, Y. F., & Kim, K. H. (2019). Heavy metals infood crops: Health risks, fate, mechanisms, and management. In EnvironmentInternational (Vol. 125, pp. 365–385). Elsevier Ltd.https://doi.org/10.1016/j.envint.2019.01.067
18. Andjelkovic, M., Djordjevic, A. B., Antonijevic, E., Antonijevic, B., Stanic, M., Kotur-Stevuljevic, J., Spasojevic-Kalimanovska, V., Jovanovic, M., Boricic, N., Wallace, D., & Bulat, Z. (2019). Toxic effect of acute cadmium and lead exposure in rat blood, liver, andkidney. International Journal of Environmental Research and Public Health, 16(2).https://doi.org/10.3390/ijerph16020274
19. Wang, Y., Tang, Y., Li, Z., Hua, Q., Wang, L., Song, X., Zou, B., Ding, M., Zhao, J., & Tang, C. (2020). Joint toxicity of a multi-heavy metal mixture and chemoprevention insprague dawley rats. International Journal of Environmental Research and PublicHealth, 17(4). https://doi.org/10.3390/ijerph17041451
20. Liu, M., Deng, P., Li, G., Liu, H., Zuo, J., Cui, W., Zhang, H., Chen, X., Yao, J., Peng,X., Peng, L., Liu, J., Zheng, W., Yan, W., & Luan, N. (2024). Neurotoxicity of CombinedExposure to the Heavy Metals (Pb and As) in Zebrafish (Danio rerio). Toxics, 12(4).https://doi.org/10.3390/toxics12040282
21. Fridovich. (1978). The Biology of Oxygen Radicals: The superoxide radical is an agent of oxygen toxicity; superoxide dismutases provide an important defense. Science,201(4359):(210504), 875–880. 10.1126/science.210504

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 19-30 | ISSN:2251-6727

22. Patra, R. C., Rautray, A. K., & Swarup, D. (2011). Oxidative stress in lead and cadmiumtoxicity and its amelioration. Veterinary Medicine International, 2011.https://doi.org/10.4061/2011/457327
23. Qu, F. & Zheng, W. (2024). Cadmium Exposure: Mechanisms and Pathways ofToxicity and Implications for Human Health. Toxics, 12(6).https://doi.org/10.3390/toxics12060388
24. Nandi, A., Yan, L.-J., Jana, C. K., & Das, N. (2019). 10.1111/J.1749-6632.2010.05603.X. Oxidative Medicine and Cellular Longevity, 2019, 1–19.
25. Lubos, E., Loscalzo, J., & Handy, D. E. (2011). Glutathione peroxidase-1 in health anddisease: From molecular mechanisms to therapeutic opportunities. Antioxidants andRedox Signaling, 15(7), 1957–1997. https://doi.org/10.1089/ars.2010.3586
26. Taourel, B. D., Guérin, M, & Torreilles, J (1992). Is malonaldehyde a valuable indicator of lipid peroxidation?Biochemical Pharmacology44, 985-988.
27. Su, L. J., Zhang, J. H., Gomez, H., Murugan, R., Hong, X., Xu, D., Jiang, F., & Peng, Z.Y. (2019). Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis,Autophagy, and Ferroptosis. Oxidative Medicine and Cellular Longevity, 2019.https://doi.org/10.1155/2019/5080843
28. Yalin, S., Comelekoglu, U., Bagis, S., Sahin, N. O., Ogenler, O., & Hatungil, R. (2006).Acute effect of single-dose cadmium treatment on lipid peroxidation and antioxidantenzymes in ovariectomized rats. Ecotoxicology and Environmental Safety, 65(1),140–144.https://doi.org/10.1016/j.ecoenv.2005.06.006
29. Poli, V., Madduru, R., Aparna, Y., Kandukuri, V., & Motireddy, S. R. (2022).Amelioration of Cadmium-Induced Oxidative Damage in Wistar Rats by Vitamin C, Zincand N-Acetylcysteine. Medical Sciences (Basel, Switzerland), 10(1).https://doi.org/10.3390/medsci10010007
30. Lushchak, V. I., Duszenko, M., Gospodaryov, D. V., & Garaschuk, O. (2021). Oxidativestress and energy metabolism in the brain: Midlife as a turning point. Antioxidants,10(11), 1–19.https://doi.org/10.3390/antiox10111715
31. Lee, K. H., Cha, M., & Lee, B. H. (2020). Neuroprotective effect of antioxidants in thebrain. International Journal of Molecular Sciences, 21(19), 129.https://doi.org/10.3390/ijms21197152
32. Wang, X. & Michaelis, E. K. (2010). Selective neuronal vulnerability to oxidativestress in the brain. Frontiers in Aging Neuroscience, 2(MAR), 1–13.https://doi.org/10.3389/fnagi.2010.00012
33. Olufunmilayo, E. O., Gerke-Duncan, M. B., & Holsinger, R. M. D. (2023). OxidativeStress and Antioxidants in Neurodegenerative Disorders. Antioxidants, 12(2), 1–30.https://doi.org/10.3390/antiox12020517
34. Hegazy, A. A., Domouky, A. M., Akmal, F., & El-wafaey, D. I. (2023b). Possible roleofselenium in ameliorating lead-induced neurotoxicity in the cerebrum of adult male rats:an experimental study. Scientific Reports, 13(1), 15715. https://doi.org/10.1038/s41598-023-42319-3
35. Gupta, R., Shukla, R. K., Pandey, A., Sharma, T., Dhuriya, Y. K., Srivastava, P., Singh,M. P., Siddiqi, M. I., Pant, A. B., & Khanna, V. K. (2018). Involvement of PKA/DARPP-32/PP1α and β- Arrestin/Akt/GSK-3β Signaling in Cadmium-Induced DA-D2 Receptor-Mediated Motor Dysfunctions: Protective Role of Quercetin. Scientific Reports, 8(1),1–18. https://doi.org/10.1038/s41598-018-20342-z