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Cadmium and Mercury Exposure: Oxidative, Neurobehavioural and Histological Alterations to the Cerebellum of Wistar Rats

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Abstract

Background: The unprecedented increase in metal exposure has been aided by modern industrialization and anthropogenic activities. Cadmium and mercury are recognized as two of the most common heavy metals with destructive impacts on most organ systems. The present study was designed to investigate and improve existing literature on the possible deleterious effects of cadmium and mercury exposure.
Methods: Adult Wistar rats were treated with cadmium chloride (5 mg/kg/day) and mercury chloride (4 mg/kg/day) for 14 days. Body, brain and cerebellar weights, motor deficits, antioxidant and lipid peroxidation activities as well as histological alterations to the cerebellum were evaluated at the end of the experiment.
Results: Findings showed a significant reduction in body and brain weights, dysregulation of antioxidant enzymes activity and impaired locomotion and exploratory activity in treated rats. Also, an increase in lipid peroxidation and degeneration of Purkinje cells of the cerebellum was observed in treated rats.
Conclusion: Overall, these results corroborate previous findings that cadmium and mercury induce deleterious effects on the cerebellum and central nervous system. In addition, this study helps to provide an anatomical perspective and information on the exact cerebellar changes induced by cadmium and mercury in Wistar rats.

Keywords: Neurotoxicity; cadmium; mercury; locomotion; exploration; cerebellum

Introduction

The toxicity of metals and their adverse effects on human health are well documented. The unprecedented increase in metal exposure has been aided by modern industrialization and anthropogenic activities such as mining, smelting and domestic as well as agricultural use of metals and metal-containing compounds.[1] Metals, known for their high density and presence all over the earth, can accumulate and adversely affect the ecosystem and biological organisms.[2] Some of these heavy metals include cadmium, cobalt, lead, mercury, aluminium, manganese, silver, uranium, vanadium, and zinc among others. Globally, a great number of people suffer from metal toxicity through water, air and food contamination. The biological activities of metals are linked to their chemical properties and their ability to react with biological systems; this occurs through the loss of one or more electrons to form metal cations with high affinity to the nucleophilic sites of essential macromolecules.[3] Following the accumulation, transportation and compartmentalization of metals into body tissues/cells, it binds to proteins and nucleic acids thereby damaging macromolecules and disrupting cellular functions.[4] Reports indicate that the resultant effects of metal toxicity include gastrointestinal, kidney and immune dysfunction, skin lesions, birth defects, cancer and nervous

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system disorders.[3]

The toxicity of heavy metals is reported to be dose-dependent; with high-dose exposures leading to greater toxicity in animals and humans.[5] Although the exact mechanisms of metal toxicity are unclear, excessive reactive oxygen species (ROS) production, antioxidant enzymes inactivation and suppression of the antioxidant defence system have been implicated.[3] As a nervous system toxicant, neurodegeneration is reported to be the most common effect of metal accumulation and toxicity. An etiological association between metal accumulation in the brain and various neurological disorders has also been reported.[6] In the brain, metal accumulation induces several damaging intracellular events such as oxidative and endoplasmic reticulum stress, apoptosis, protein misfolding, autophagy dysregulation, mitochondrial dysfunction and DNA fragmentation.[7] The dysregulation of these intracellular events is known to cause neurodegeneration and other nervous system disorders, which are mostly manifested as cognitive, learning and memory impairments, as well as locomotion disorders.

Although available reports show the toxicity of heavy metals, there is a dearth of relevant research evidence to demonstrate the neurobehavioural and histological changes to the cerebellum following exposure to heavy metals. Accordingly, the present study sought to examine the neurotoxic effects of cadmium and mercury exposure in Wistar rats using body, brain and cerebellar weight changes as well as neurobehavioral, biochemical and histological assessments, thus leading to a better understanding of metal poisonings and their management.

Materials and methods

Experimental animals: Eighteen (18) adult Wistar rats weighing between 150 – 175g were obtained from the Department of Anatomy Animal House, University of Benin, Edo State, Nigeria. Following appropriate weighing and labelling, the rats were kept in wooden cages and acclimatized for two weeks. The experiments were carried out in the Department of Anatomy, School of Basic Medical Sciences, University of Benin and rats were fed with standard rat chow (Bendel livestock feed, Edo state, Nigeria) and water throughout the entire study period. Ethical approval was granted by the Research Ethics Committee of the College of Medical Sciences, University of Benin, with the number CMS|REC|2021|165.

Chemicals and reagents: Normal saline was manufactured by Unique Pharmaceuticals, Sango-Otta, Nigeria. Mercury Chloride ($HgCl_2$, 99% purity) and Cadmium Chloride ($CdCl_2$, 98% purity) by Loba Chemie Pvt. Ltd, Mumbai, India. Other reagents were all of the analytical grades.

Administration of experimental drugs: 0.5g of Mercury chloride and cadmium chloride were dispersed in 10mL of distilled water respectively and administered via oral intubation. Dosages were calculated per body weight of animals according to the formula below, as previously reported.[8]

$$\text{Volume (mL)} = \frac{\text{Dose (g/kg)} \times \text{Bodyweight (kg)}}{\text{Concentration (g/mL)}}$$

Experimental Design: Adult Wistar rats were weighed, marked and randomly divided into three groups of six animals each.

Table 1: Treatment regimen across experimental groups

GROUPS	
A – Control	served as control, received distilled water only
B – Cd	received $CdCl_2$ (5 mg/kg body wt.) for 14 days
C – Hg	received $HgCl_2$ (4 mg/kg body wt.) for 14 days

Determination of Neurobehavioural activity (Open Field Test): This was done to evaluate the exploratory motor function and locomotor activity, as previously described.[9] Each rat was placed in the centre of a square wooden arena (72 cm × 72 cm × 20 cm) with lines on its floor dividing it into 18cm by 18 cm square. This test was done on the 15th day and experimental rats were given the freedom to fully explore the open field arena for 5 min. With a video camera mounted directly above the open field apparatus, activities such as number of line crossings (number of segments crossed with the four paws), rearing (frequency with which the rat stood on their hind legs in the arena), freezing (time

with which the rat was completely stationary) and number of fecal pellets were recorded.

Determination of Relative Brain weight/Organo-somatic index: The body weight of each rat was recorded before and after treatment (day 15). At the end of the experiment, rats were sacrificed by cervical dislocation and the brains were removed immediately by opening the cranial cavity. Thereafter, the whole brain and cerebellar weights of rats across experimental groups were recorded. To mitigate the individual bodyweight differences, the organo-somatic index was calculated and expressed as a percentage of the final body weight at sacrifice.[10] The cerebellum was separated and processed for biochemical and histological investigations.

Evaluation of biochemical parameters: Following sacrifice, the cerebella were subjected to the following estimations as previously reported. (i) Superoxide Dismutase (SOD), based on auto-oxidation of adrenaline,[11] (ii) Catalase (CAT),[12] (iii) Malondialdehyde (MDA), using the thiobarbituric acid assay[13] and (iv) Glutathione Peroxidase (GPx), based on the oxidation of pyrogallol to purpurogallin by peroxidase activity.[14]

Histological evaluation: The cerebella of experimental rats were fixed in Bouin's fluid for 72 hours and processed through the paraffin wax embedding method as previously reported.[15] The Haematoxylin and Eosin staining method described by Drury and Wallington was also performed.[15] The processed slides were viewed and captured with a LABO® research trinocular microscope (Labo Microsystems GmbH, Germany) mounted on an Omax 9.0MP USB Digital Microscope Camera (Korea).

Statistical Analysis: Obtained data were analyzed using GraphPad Prism Software V7 (www.graphpad.com/scientific-software/prism/). Results were presented as mean ± standard error of mean. One-way analysis of variance (ANOVA) followed by the Tukey multiple comparisons was used to determine statistical significance ($p<0.05$).

Results

Effect of treatment on body and brain weights

The findings from the body, brain and cerebellar weight across experimental groups are presented in Table 2. A significant decrease ($p< 0.05$) was observed in the final body and absolute whole brain weight of rats treated with cadmium and mercury when compared to control. Also, there was a significant decrease ($p< 0.05$) in the relative brain weight/organo-somatic index of rats treated with mercury when compared to control.

Table 2: Effects of Cadmium and Mercury on Body, Brain, Cerebellum weights and Relative Brain weight/Organo-somatic index across experimental groups after 14 days.

Groups	Initial Body weight (g)	Final Body weight (g)	Absolute whole brain weight (g)	Cerebellar weight (g)	Relative brain weight (%)	Relative cerebellar weight (%)	Cerebellum/ Brain weight ratio
Control	164.0 ± 3.606	180.3 ± 4.096	1.743 ± 0.034	0.6133 + 0.0186	0.9667 ± 0.0067	0.3400 ± 0.0032	0.3517 ± 0.0037
Cd	159.3 ± 5.132	166.0 ± 2.517*	1.587 ± 0.021*	0.5400 + 0.0231	0.9567 ± 0.0088	0.3250 ± 0.0103	0.3413 ± 0.0092
Hg	158.3 ± 2.333	164.0 ± 2.309*	1.540 ± 0.029*	0.5233 + 0.0240	0.9333 ± 0.0033*	0.3187 ± 0.0095	0.3393 ± 0.0106

Values are given as mean ± SEM of each group. * $p<0.05$ compared with the control group

Effect of treatment on Neurobehavioural activity

The findings from the Open Field Test (OFT) evaluation are presented in Table 3. A significant decrease ($p< 0.05$) was observed in line crossing and frequency of rearing activity in rats treated with cadmium and mercury when compared to control. Similarly, a significant increase ($p< 0.05$) in freezing was observed in rats treated with cadmium and mercury when compared to control. For the number of fecal pellets, no significant difference ($p> 0.05$) was observed between treated and control.

Table 3: Effects of cadmium and mercury on locomotion and exploration activity across experimental groups after 14 days.

Groups	Line crossing	Freezing (secs)	Frequency of rearing	Fecal Pellets
Control	13.67 ± 1.4530	106.7 ± 13.02	7.667 ± 0.8819	0.333 ± 0.3333
Cd	5.667 ± 1.2020*	198.3 ± 20.02*	3.333 ± 0.8819*	1.667 ± 0.3333
Hg	4.333 ± 0.8819*	205.0 ± 15.04*	2.667 ± 0.8819*	2.000 ± 0.5774

Values are given as mean ± SEM of each group. * $p<0.05$ compared with the control group

Effect of treatments on Antioxidant and MDA Activity

The findings from the activity of antioxidants and MDA in the cerebella across experimental groups are presented in Table 4. A significant decrease ($p<0.05$) in cerebellar SOD, CAT and GPx were observed in the cadmium and mercury treated groups when compared to control. Similarly, a significant increase ($p<0.05$) in MDA was observed in the cadmium and mercury treated groups when compared to control.

Table 4: Effects of cadmium and mercury on antioxidant and MDA activity across experimental groups after 14 days.

Groups	SOD (U/ml)	CAT (U/ml)	GPx (U/ml)	MDA (mmol/ml)
Control	6.857 ± 0.2106	165.7 ± 3.4800	147.9 ± 4.0070	24.14 + 1.2190
Cd	3.560 ± 0.3151*	134.7 ± 2.8810*	131.6 ± 0.9783*	42.40 + 0.7549*
Hg	3.500 ± 0.2774*	141.7 ± 4.4680*	127.7 ± 0.5476*	42.31 + 1.9410*

Values are given as mean ± SEM of each group. * $p<0.05$ compared with the control group

Effect of treatments on the histology of the cerebellum

The Haematoxylin and Eosin stained sections of the cerebellum were evaluated under light microscopy (Figure 1A-C). As shown in plate A, the three layers (molecular layer, Purkinje's cell layer, and granular cell layer) are demonstrated in control rats. Following treatment of rats with cadmium and mercury for 14 days, a disruption of the normal architecture of the cerebellum was observed. These alterations were demonstrated by large spaces in between the Purkinje's cell layer and the molecular layer or granular layer, signifying depletion and degeneration of the Purkinje cells (Plate B and C). Also observed are degenerating cells in the molecular layer of the cerebellum of rats treated with cadmium and mercury.

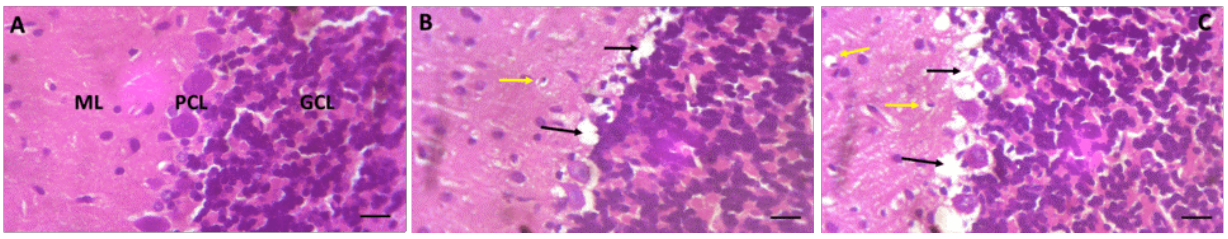

Figure 1: Transverse section of the cerebellum across experimental groups (A) Control group showing a normal histological structure of cerebellum layers. (B & C) Cd and Hg treated groups showed severe vacuolation and depletion of the cells in the Purkinje layer (black arrows) and degenerating cells in the molecular layer (yellow arrows). (H&E x400). Scale bar: 25µm. ML: Molecular layer; PCL: Purkinje cell layer: GCL: Granule cell layer.

Discussion

Cadmium and mercury are recognized as one of the most common heavy metals with destructive impacts on most organ systems. In this study, the impact of metal pollutants (cadmium and mercury) on Wistar rats was evaluated. Findings showed that exposure to cadmium and mercury negatively affected the body and brain weight of rats when compared to control. Reports indicate that changes in body weight are often considered useful markers for determining the animal's health status, and a decrease in body weight highlights the health deterioration of animals during the experimental period.[16,17] Similarly, the most sensitive indicator of toxicity is considered to be negative changes in organ weight which often heralds morphological alterations and organ atrophy/damage.[18] This loss in body and organ weight could be a result of elevated lipid and protein degeneration induced by cadmium and mercury. These findings agree with other reports showing that heavy metals such as cadmium and mercury cause disturbances in the total body and organ weights of rats possibly due to reduced water and food consumption, tissue injury and impaired organ function.[19,20] Diminished cerebellar weight due to the toxicity of heavy metals has also been previously reported.[21,22]

The Open field test is often utilized to evaluate anxiety, locomotion and exploratory activity.[23] In this test, locomotion activity is commonly measured by the number of lines crossed, freezing and frequency of rearing while the number of fecal pellets is an indicator of anxiety. Although reports show that elevated rearing and line crossing demonstrates increased locomotor and exploratory activity, an increase in freezing and fecal pellets indicates impaired locomotion and elevated anxiety respectively.[24] In this study, impaired locomotion and exploration were observed in rats exposed to cadmium and mercury when compared to control. This is in agreement with previous findings demonstrating that the areas of the brain involving motor control and coordination are affected by heavy metal toxicity.[25-27]

Dysregulation of the oxidative status, via excessive production of oxidants or impairment of antioxidant activity, is often considered one of the consequences of heavy metal toxicity and poisoning in animals and humans.[28] Antioxidant molecules (endogenous or exogenous) and enzymes (SOD, CAT and GPx) play critical roles in attenuating the debilitating effects of oxidative stress.[29] Cadmium and mercury are known to induce potent oxidative stress through excessive generation of reactive oxygen species, which is responsible for its toxic effects. Functionally, antioxidants scavenge free radicals and protect against or ameliorate the damages induced by excessive generation of reactive oxygen species, thus improving immunity and minimizing the risk for the development of neurodegenerative disorders.[30] The reduction of glutathione and the impairment of SOD and CAT activities have been implicated in the lipid peroxidation process, often considered a key event in heavy metal toxicity.[31] From this study, it is observed that cadmium and mercury induced oxidative stress in experimental rats; this is demonstrated by the increase in MDA (lipid peroxidation marker) and decrease in SOD, CAT and GPx activities when compared with control. These findings indicate that the concentrations of cadmium and mercury affected normal cellular functions, inhibited SOD, CAT and GPx activities and induced lipid peroxidation in experimental rats. This corroborates previous findings demonstrating the oxidative damaging effects of cadmium and mercury.[32,33]

Proper coordination of cognitive function, locomotion and exploratory behaviour is dependent on the integrity of the nervous system.[34] Studies show that the cerebellum, a delicate anatomical structure responsible for the coordination and regulation of motor and non-motor functions, is highly susceptible to intoxication and poisoning. Impairments to motor coordination and balance occur due to lesions on the cerebellum, and the Purkinje cells are regarded as the most vulnerable to damage following exposure to heavy metals.[21,35] In

the present study, several histological alterations were observed in the cerebella of cadmium and mercury treated rats. These included large spaces in between the Purkinje cell layer and the molecular layer or granular layer, signifying marked depletion and degeneration of Purkinje cells in the Purkinje layer together with degenerating cells in the molecular layer of the cerebellum. These findings are in agreement with previous studies demonstrating alterations to cerebellar architecture, particularly the Purkinje cells owing to the sensitivity of the Purkinje cell layer, following heavy metal exposure.[21,22,36,37]

In conclusion, the results from this study highlight oxidative stress as a possible mechanism of action through which cadmium and mercury impair motor function and alter cerebellar architecture. Although this study reveals the exact cerebellar alterations induced by cadmium and mercury, the findings also highlight the dysregulation of antioxidant enzymes activity in the cerebellum. Consequently, neuroprotective and therapeutic strategies aimed at enhancing proper regulation and protection of antioxidants may be useful in the prevention, management and/or treatment of neurological disorders linked to heavy metal exposure.

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