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Original Research

Lactational exposure to cadmium induced alterations in the hematological indices and the oxidative status in brain, liver and testes of rat pups

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Abstract
Hematology, antioxidant, and certain biochemical indices in brain, liver, and testicular tissues of 30 male rat off-springs were studied after lactational cadmium exposure. Ten lactating Sprague–Dawley females received either 0 ppm (control) or 20 ppm cadmium chloride in their drinking water during the lactational period. On day 24, the male pups were weaned and sacrificed. Distortion of hemopoietic features such as decrease in red blood cells count, hemoglobin, hematocrit, and platelet values were seen in the exposed pups. Increased lipid peroxidation and depressed antioxidant defense levels in brain, liver and testes of the exposed rat pups were observed. Serum activities of alkaline phosphatase and lactate dehydrogenase showed a significant increase, whereas a reduction was observed in the level of testosterone hormone in exposed pups. Cadmium induced neuronal degeneration, necrosis in hepatocytes, and degeneration in seminiferous tubules. These findings indicated that lactational exposure to cadmium can disrupt several organ functions in newborn rat pups. Therefore, the sources of cadmium exposure must be restricted and regularly monitored in the environment

INTRODUCTION

Cadmium (Cd) is a biotoxic environmental pollutant that accumulates in body tissues such as the lungs, liver, kidneys, bones, reproductive organs and immune system [1, 2]. The toxic effects of Cd on organisms include nephrotoxicity, carcinoegenicity, teratogenicity and endocrine disruption [3].

Cd is transferred from a lactating dam to a suckling in low concentrations via milk [4]. Studies have shown that a considerable fraction of the administered cadmium is sequestered in the mammary glands of lactating rodents. Although relatively low, the transfer of Cd through maternal milk represents the primary route of offspring exposure when rodents are exposed during gestational and lactational periods [5]. Moreover, Cd levels in maternal milk are correlated with the degree of maternal exposure [5]. Several studies have shown that the absorption and retention of orally administered Cd in a newborn rodent is affected by dietary composition and that milk enhances absorption [6-8].

It has been demonstrated that Cd can be concentrated in the cell nucleus, thus perturbing cell proliferation and DNA synthesis [9, 10]. Therefore, this metal could affect the germ cells of pups during pre- and postnatal development. Cd may also cause the deterioration of cell membranes by binding to metalothionein (MT) or glutathione and may consequently interfere with the ability of these proteins to avoid oxidative stress [11]. In addition, Cd can also replace essential metals such as copper and zinc in several metalloproteins, altering the protein conformation and affecting their activity because this element interacts ubiquitously with sulphydryl groups of amino acids, proteins and

enzymes [12, 13].

Cd can cause oxidative stress through several mechanisms: the Fenton reaction [14], the depletion of cellular glutathione, alterations in the mitochondrial electron transfer chain [15] and the inhibition of antioxidant enzymes [16]. These changes may result in biochemical and morphological alterations in the affected organs.

Gestational and lactational exposure to Cd leads to neurotoxicity and neurobehavioral changes. Such changes are observed in the developing brain in the hippocampal region. This region is associated with increased lipid peroxidation, a surge in reactive oxygen species (ROS) and depressed antioxidant defense [17]. The brain growth spurt occurs postnatally in rats, with a maximum rate of growth occurring at about 10 days of age (i.e. during the suckling period). During this period, lipid levels increase rapidly in the brain [18]. It could be hypothesized that the effects of Cd on the developing brain may be mediated by an effect on the fatty acid composition in milk and in the brain of the suckling offspring. Some reports show that Cd may interact with lipid metabolism. Generally, Cd enhances lipid peroxidation in vitro and in vivo [14]. Effects on phospholipid composition have been reported in peritoneal macrophages from mice that were chronically exposed to Cd [19], in the brains of pre- and postnatally Cd-exposed rats [20, 21], and in the liver cells of rats after in vitro and in vivo treatment with Cd [22-24]. Liver is the main tissue where both the metabolism and catabolism of fatty acids takes place. It is known that Cd exposure may lead to hepatic lipid peroxidation in rodents [25].

Several previous studies have found that Cd [26] strikingly alters the testes of adult rats and mice. The oral administration of Cd to rodents results in necrosis, testicular atrophy, and sterility in males [27]. Gestational exposure to Cd has been shown to induce biochemical and reproductive effects with alterations in testicular steroidogenesis and the antioxidant system of cauda-epididymis [28].

The objective of the present study was to concentrate on the expected different toxic effects of neonatal exposure to environmentally relevant levels of Cd only through maternal milk. In contrary, the most of the previous studies were concerned with two types of exposure together. A concentration of 20 ppm Cd in a dam's drinking water was selected as representative of the maximum levels of Cd found in food and water.[29]

MATERIAL AND METHODS

Chemicals

Cadmium Chloride (CdCl2) was purchased from Sigma Chemical Co (St. Louis, Mo., USA).). Superoxide dismutase (SOD), and lipid peroxide malondialdehyde (MDA) were measured using commercial test kits supplied by Bio-diagnostics (Bio-diagnostics, Cairo, Egypt). All other chemicals were of the highest grade available commercially.

Experimental Animals and Exposure Design:

Ten pregnant female Sprague–Dawley rats, purchased 1 week prior to parturition from the Animal Laboratory House of Assiut University, Assiut, Egypt. Female rats were housed in a pathogen-free animal facility, and cared for in compliance with the ethical guidelines prescribed by the Institution for the Animal Care. Within 12 h of birth (day 1), male pups were removed from their mothers, sexed and randomly re-assigned to lactating females so as to provide three male pups per female. These lactating females received either 0 ppm (control) or 20 ppm Cd as (CdCl2) in their drinking water. The drinking water was administered *ad libitum* to female rats, as distilled water in control group, or CdCl2 solution prepared in distilled water in exposed group, and replaced daily to minimize Cd precipitates. Pups were weaned on day 24. The dose of Cd applied in the present study is similar to the Cd dose reported, in the Toxicological Profile for Cd by the U.S. Department of Health & Human Services, to produce effects on normal development in rats [29].

Sample collection

On days 24 total of 30 male rat pups were weighed and euthanized by CO2 asphyxiation. The whole blood was collected by heart puncture into tubes containing EDTA for different hematological parameters. A second blood fraction was collected without anticoagulant and centrifuged at 4000X g for 10 min for serum separation. Brain, liver and testes were immediately excised and kept frozen in liquid nitrogen and stored at −80 ◦C for oxidative status measurements. Some specimens of brain, liver and testes were randomly selected, fixed in 10% neutral buffer formalin and processed for histopathological studies.

Hematological parameters:

Hematological parameters [Red Blood Corpuscles (RBC), Hemoglobin (HB), Hematocrite (HCT), Red Blood cell distribution width (RDW), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Corpuscles (WBC) and Differential Leucocytic levels]. Thrombocytic indices as [Total Platelet Count (PLT), Mean Platelet Volume (MPV), Total Platelet Crit (PCT) and Platelet Distribution Width (PDW)] were analyzed by automated parameter hematology analyzer (MICROS 60-Abx Diagnostics, Montpellier, France).

Lipid peroxidation assay

Measurement of malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) have been used as an indicator of lipid peroxidation. MDA and 4HNE were estimated by the method of Buege and Aust [30]. Briefly 200 ml aliquot of brain, liver and testes homogenates (10% w/v in Tris–HCl buffer, 20 mM, pH 7.4) was transferred to 650 ll of 10.3 mM 1-methyl-2-phenylindole in acetonitrile and vortex mixed. To assay MDA + 4HNE, 150 ml of 15.4 M methanesulfonic acid was added, vortexed and incubated at 45 0C for 40 min. To assay MDA alone, 150 ml of 37% HCl was added instead of methanesulfonic acid, vortexed, incubated at 45 0C for 60 min. After incubation, samples were kept on ice, centrifuged at 9500g for 5 min and absorbance was measured at 586 nm. The levels of MDA and 4HNE are expressed as nmol of reactive substance formed/min/mg protein.

Superoxide dismutase (SOD) activity assay

Changes in brain, liver and testes Cu/Zn superoxide dismutase (Cu/Zn-SOD) activity were analysed using the Bioxytech SOD-525 spectrophotometric assay kit. Briefly, tissues homogenate from brain, liver and testes were prepared according to the protocol described in the kit. Spectrophotometric assay of SOD activity was based on the enzyme's ability to inhibit superoxide-driven NADH oxidation. The rate of reaction was measured by recording to the change in the absorbance at 550 nm. The activity was expressed as units per gram protein in tissues [31].

Protein assay

Protein concentrations were measured by the method of Bradford [32], using bovine serum albumin as a standard. Protein concentration used in the concentration of SOD and MDA & HAE can be expressed as activity per mg of protein by dividing the units /ml of protein concentration.

Measurement of testosterone

Serum testosterone levels were measured by RIA following extraction with diethyl ether as described previously [33].

Measurement of Alakaline phosphatase and Lactate dehydrogenase

Serum alkaline phosphatase and Lactate dehydrogenase (LDH) were determined colormetrically according to Vassaultt [34] and Tietz [35], respectively.

Histopathological study

Brain, liver and testes samples were dissected and fixed in 10% neutral formalin, dehydrated in ascending grades of alcohol and imbedded in paraffin wax. Paraffin sections (5 μm thick) were stained for routine histological study using haematoxylin and eosin (H&E).

Statistical analysis

For continuous exposure and outcome variables means and standard deviations (SD) were computed using SPSS 11.0 for Windows. Data are expressed as means ± SD. Statistical analysis was performed to compare treated groups with respective control groups using one-way analysis of variance (ANOVA), followed by the Duncan's multiple range test when appropriate. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Hematological determination

A significant (p <0.05) decrease in RBC, Hg, HCT, RDW, MCV, MCH and MCHC was obtained in rat pups exposed to CdCl2 through maternal milk in comparison with the control pups (Table 1). And also, a significant decrease in terms of PLT and PDW values was observed in the same rat group. There is no significant difference was observed in MPV and PCT values when compared with the control pups (Table 2).

Table 1. Changes in the different hematological indices after the lactational exposure to cadmium chloride in rat pups.

	RBCs 106/ml	Hg g/dl	HCT %	RDW %	MCV um	MCH pg	MCHC g/dl
Exposed Pups	7.3±0.4*	13.9±0.7*	38.8±4.2*	26.9±0.3*	46.3 ±2.1*	18.2±0.4*	33.2±0.3*
Control Pups	8.7±0.3	15.0±0.5	49.3±0.6	24.5±0.4	48.7 ±2.5	22.8±1.7	37.4±2.0

Data are expressed as means ± S.D. of n= 30 rat pups per group.*denotes $P < 0.05$ as compared to control group (One- way ANOVA/Duncan).

Table 2. Changes in the thrombocytic indices after the lactational exposure to cadmium chloride in rat pups.

	PLT 10^3 /ml	MPV um	PDW um	PCT %	LPCR %
Exposed Pups	710.4±91.3*	6.20±0.22	8.95±0.39*	0.42±0.04	6.72±1.1
Control Pups	762.7±14.8	6.48±0.38	21.29±2.95	0.43±0.01	7.48±0.6

Data are expressed as means ± S.D. of n= 30 rat pups per group.*denotes P < 0.05 as compared to control group (One- way ANOVA/Duncan).

Table 3. Changes in the oxidative status after the lactational exposure to cadmium chloride in rat pups.

	SOD IU/mg protein	**MDA&HAE** nmol/mg protein
Exposed Pups Brain	0.36 ±0.02*	5.00±0.16*
Control Brain	0.98 ±0.14	3.50±0.31
Exposed Pups Liver	0.66 ±0.01*	7.60±0.16*
Control Liver	0.133 ±0.11	4.10±0.31
Exposed Pups Testes	0.52 ±0.04*	6.40±0.16*
Control Testes	0. 93 ±0.10	3.90±0.31

Data are expressed as means ± S.D. of n= 30 rat pups per group.*denotes *P* < 0.05 as compared to control group (One-way ANOVA/Duncan).

Table 4. Changes in the serum levels of alkaline phosphatase, lactate dehydrogenase and testosterone hormone after the lactational exposure to cadmium chloride in rat pups.

	ALP U\l	**LDH** mM/L	**Testosterone** ng/L
Exposed Pups	85.1±10*	0.36 ±0.02*	2.0 ±0.2*
Control Pups	50.4±3	0.98 ±0.14	3.3 ± 0.3

Data are expressed as means ± S.D. of n= 30 rat pups per group.*denotes *P* < 0.05 as compared to control group (One-way ANOVA/Duncan).

Oxidative status in brain, liver and testes

The levels of LPO were significantly elevated in the Cd -exposed rat pups brains, livers and testes compared to the rats in the control group. The Cd-exposed rat pups showed a marked reduction (p <0.05) in the activities of SOD in the brain, liver and testes as compared to rat pups in the control group (Table 3).

Biochemical parameters

A significant (p <0.05) reduction in testosterone hormone concentration (g\dl) was observed in the serum of the Cd- exposed rate pups than in the control pups. A significant (p <0.05) elevation in ALP and LDH concentration was recorded in the serum of the Cd- exposed rate pups than in rats in the control group (Table. 4).

Histopathology

Brain

The brains of the control, untreated rat pups showed normal neurons of the cerebral cortex (Fig. 1a). Meanwhile, the brains of rat pups exposed to Cd through lactation were macroscopically slightly congested**.** Microscopically, brain sections of rat pups revealed neuronal degeneration, pyknosis of neurons, central chromatolysis of the nucleus and cytoplasmic vaculation (Fig.1b). Moreover, brain of rat pups showed necrosis of neurons with shrinkage and margination of the nucleus associated with perineural gliosis and astrocytosis (Fig.1c).

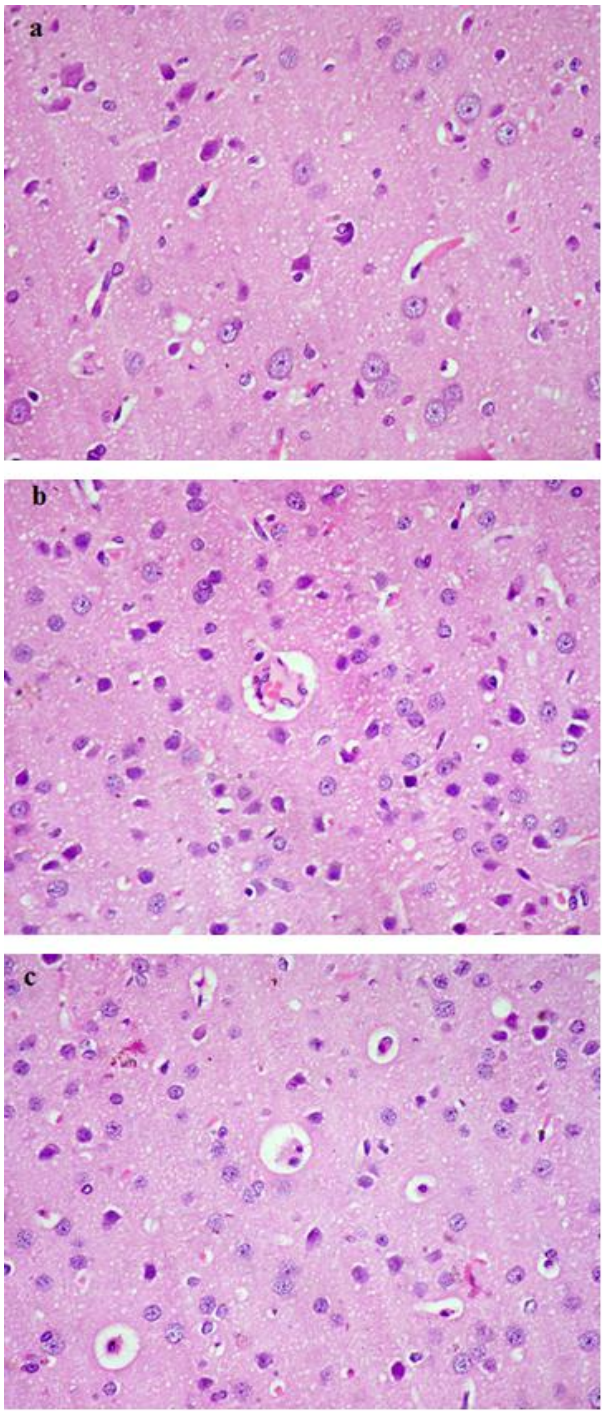

Fig 1. Sections of the brain from control and rat pups exposed to CdCl2 via lactation, showing neurons and perineural tissues of the cerebral cortex. **a)** Normal cerebral cortex neurons in the control. H&E. X40. **b)** Central chromatolysis of the nucleus and cytoplasmic vaculation in cerebral cortex neurons H&E. X40. **c)** Shrinkage and margination of the nucleus of cerebral cortex neurons associated with perineural gliosis and astrocytosis H&E. X40.

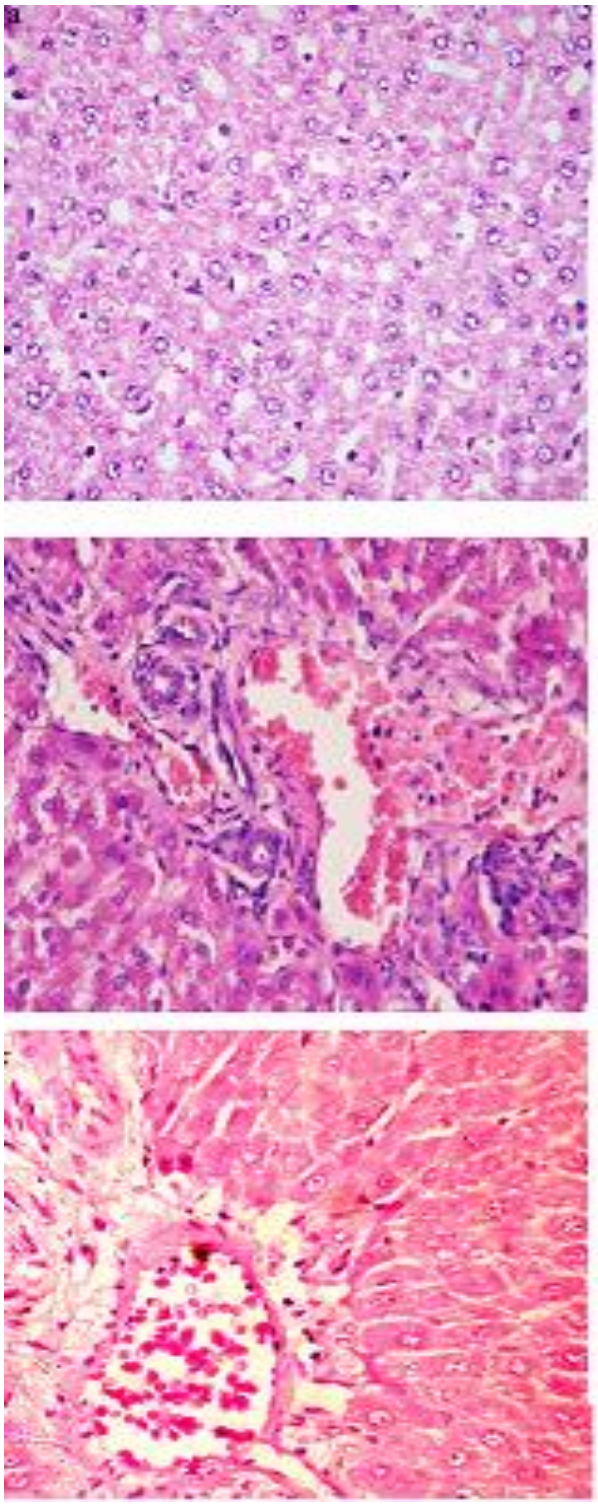

Fig 2. Sections of rat pup livers exposed to CdCl2 via lactation. a) Hepatic cells showing vacuolar and fatty degeneration H&E. X25. **b)** Congested blood vessel with cellular infiltration H&E. X40. **c)** Portal area showing an increase in kupffer cell infiltration H&E. X40.

Liver

Blurred trabecular structure, vacuolar degeneration and increased density of nuclear chromatin with very compact nuclear structure were found in hepatocytes (Fig.2a). Moreover, congested blood vessel with mononuclear cell infiltrations and necrosis of single cells were evident (Fig.2b). The portal area showed high density of kupffer cell infiltration (Fig.2c).

Testes

In the testes, Cd caused damage to the histology of the testes. These damages were characterized by destruction of germ cells and semniferous tubules, vascular congestion, focal necrosis of tissue, reduction of spermatocytes, and pyknosis associated with destruction of nucleus (Fig. 3a). There is edema in the seminiferous tubules and interstitial tissue (Fig. 3b).

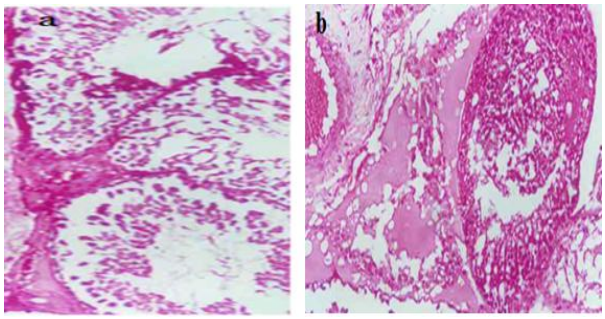

Fig 3. Sections of rat pup testicles exposed to CdCl2 via lactation **a)** Seminiferous tubules showing degeneration and nuclear pyknosis in spermatocytes H&E X40. **b)** There is an extensive interstitial edema among the seminiferous tubules H&E X40

DISCUSSION

The toxic effects of Cd on adult rats are well documented, whereas only a few studies with administration of Cd via lactational exposure alone have been conducted in newborn rats [7]. The results obtained in this study showed that newborn rats exposed to Cd through maternal milk had a distortion of hemopoietic features in the form of lower RBC count, Hb concentration, and Ht value. These findings indicate treatment with Cd induces anemia in rat pups. Several studies mentioned that oral dietary supplementation with Cd induced toxic effects on hematological indices of albino rats [36]. Gestational and lactational exposure to Cd induce metabolic changes in the fetus, resulting in reduced Ht values [37] and inhibition of zinc-dependent enzymes [38]. It is known that the presence of Cd in the organism decreases the levels of iron in the blood [39] and causes a decrease in Hb concentrations. The decrease of Ht value in hemolyzed plasma of rats exposed to Cd indicates the increased destruction of erythrocytes [40, 41]. Moreover, Cd may inhibit heme synthesis by decreasing the absorption of iron from the

gastrointestinal tract [41].

Cd induces oxidative damage in different tissues by enhancing the peroxidation of membrane lipids and by inhibiting endogenous antioxidants and enzymes involved in the utilization of reactive oxygen species [42]. In the present study, the brain, liver, and testes of neonatal rats exposed to Cd by lactation indicate the presence of oxidative stress, which evidenced by an elevation in lipid peroxidation (MDA) and a decrease in antioxidant enzymes (SOD) compared to those of controls. In general, the mechanisms by which Cd can induce oxidative stress through free radicals over the production and disruption of the mitochondrial membrane which appear to be the primary target to its cellular effect [43].

Histopathological examination of the brain of neonatal rats exposed to Cd via lactational exposure revealed massive damage to the hippocampus and cerebral cortex, in the form of cellular atrophy, shrinkage, cellular necrosis, cerebral hemorrhage and cerebral edema. Neurochemical disturbances in the serotonergic system have been demonstrated during lactation in the offspring of rats exposed to low levels of Cd in the drinking water [44]. Cd induces oxidative stress in neuronal cells, which leads to protein damage [45] and subsequently induces neurodegeneration [46, 47]. These changes may be attributed to the fact that Cd is a neurotoxic metal, which induces cellular damage and oxidative stress in the brain via the overproduction of free radicals [48]. These studies are in agreement with our observations that the exposure of rats to Cd via lactation induced oxidative damage in the brain, as previously discussed. Moreover, Mukherjee et al. [17] documented that dietary exposure to cadmium, even at lower doses, can lead to free radical-induced neurotoxicity, neurobehavioral changes and alterations to neurotransmitters, and such changes are likely to be more pronounced in the developing brain due to incomplete formation of the blood brain barrier **.**

In the present study, mononuclear cell infiltration and hepatocyte necrosis were evident in the liver of neonatal of rat pups whose mothers were exposed to Cd. Moreover, a significant elevation in the serum concentrations of ALP and LDH were recorded in rat pups with lactational exposure to Cd. Cd-induced LDH release suggests that a necrotic process occurred [49]. Furthermore, Cd-induced necrosis accompanied by LDH release has been observed in several cell types [50, 51]. Generally, the liver is one of the critical target organs after both acute and chronic exposure to Cd [52]. Cd can induce lipid peroxidation in tissues [53], which may lead to necrosis, and Cd-induced liver necrosis caused the release of abnormal quantities of alkaline phosphatase and aminotransferases enzymes into the blood [54]. Free radical-induced oxidative stress causes membrane lipid peroxidation [55] may result in tissue damage and leakage of enzymes. Consequently, the elevated plasma ALT and AST along with reduction in enzymes in the liver of rats fed Cd is probably an indication of liver damage occasioned by lipid peroxidation [56].

In the current study, a significant reduction in testosterone hormone concentration in the serum of exposed rate pups was obtained, and this was accompanied by damage to the histology of the testes. This damage was characterised by the destruction of germ cells and seminiferous tubules. The toxic effects of gestational and lactational exposure to Cd on testicular steroidogenesis, the antioxidant system and male accessory gland functions attributed to disturbances in the biochemical mechanisms involved in endocrine disruptions [28]. Amara et al. [57] credited decreased testicular growth rate and plasma testosterone to Cd-induced oxidative stress and as a concurrent reduction in glutathione peroxidase, catalase, mitochondrial Mn–SOD and cytosolic CuZn–SOD, as well as increased malondialdehyde. Cd-induced oxidative stress in testicular tissues was observed in this study in the form of a decrease in antioxidant enzyme SOD activity and an increase in the levels of MDA lipid peroxidation product.

Our study concerned mainly with the exposure to Cd via lactation to determine the changes in suckling development. In contrast to other studies focused on both gestational and lactational exposure to cadmium. Results from our study and the literature suggest that cadmium intoxication exhibits different developmental defects depending on time and route of exposure, dose ingested, and age of individuals. This study indicates that alteration in neonatal development is a target for toxicity of environmental pollutants as Cd through the maternal milk. However, the precise mechanism of action needs to be further investigated.

In conclusion, lactational exposure to Cd can induce several alterations in new offspring in the form of anaemia, liver, brain and testicular damage. Moreover, these changes are mainly associated with the presence of oxidative damage indices.

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