Sjournals
Scientific Journals
Scientific Journal of Veterinary Advances (2012) 1(3) 70-81

Contents lists available at Sjournals
Scientific Journal of Veterinary advances
Journal homepage: www.Sjournals.com

Original article

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

E.E. Elsharkawy[a,*], N.A. El-Nisr[b]

[a]*Department of forensic medicine and toxicology faculty of veterinary medicine. Assuit University, Egypt.*
[b]*Animal Health Institute of Research- Egypt.*

[*]Corresponding author; Department of forensic medicine and toxicology faculty of veterinary medicine. Assuit University, Egypt.

ARTICLE INFO

Article history:
Received 31 August 2012
Accepted 15 September 2012
Available online 29 September 2012

Keywords:
Lactational cadmium
Platelets
Antioxidant system
Lactate dehydrogenase
Testosterone
Cerebral cortex

ABSTRACT

This study was carried out to investigate the effect of lactational exposure to cadmium (Cd) on suckling male rat pups. Hematological parameters, antioxidant status, some biochemical and pathophysiological indices in brain, liver and testicular tissues of rat offspring were studied. Lactating Sprague–Dawley females received either 0 ppb (control) or 20 ppm Cd as CdCl2 in their drinking water during the lactational period. Suckling male rat pups were weaned and sacrificed on day 24 for performing various biochemical assays. Distortion of the hemopoietic features as decrease in RBCs count, hemoglobin, hematocrit and platelet values were seen in exposed rat pups. Increased lipid peroxidation malondialdehyde (MDA) and depressed antioxidant defense superoxide dimutase (SOD) levels in brain, liver and testes of exposed rat pups were obtained. Serum activities of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) showed a significant increase, whereas a reduction in the level of testosterone hormone was obtained in cadmium exposed pups. In addition, Cd induces neuronal degeneration, necrosis in hepatocytes and degeneration in semineferous tubules along with interstitial edema. The previous findings are indicative of multiple targets of cadmium to disrupt several organ functions in newly borne rat pups on lactational exposure.

1. Introduction

Cadmium is a biotoxic environmental pollutant which accumulates in the body tissues such as the lungs, liver, kidneys, bones, reproductive organs and the immune system (Egwurugwu et al. 2007, Sofyan et al. 2007). The toxic effects of cadmium on organisms include nephrotoxicity, carcinoegenicity, teratogenicity and endocrine disruption (Serafim and Bebianno 2007). Cd is transferred from the lactating dam to the suckling in low concentrations via milk (Bhattacharyya, 1983). It has been 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 both gestational and lactational periods (Petersson Graw´e and Oskarsson, 2000). Moreover, Cd levels in maternal milk are correlated with the degree of maternal exposure (Petersson Graw´e and Oskarsson 2000). Several studies have shown that the absorption and retention of orally administered Cd in the new-born is affected by dietary composition and that milk enhances absorption (Eklund et al., 2001, 2004; Saric et al., 2002). It has been demonstrated that Cd can be concentrated in the cell nucleus, thus perturbing cell proliferation and DNA synthesis (Gerber et al., 1980; Coogan et al., 1994). 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 consequently interfere with the ability of these proteins to avoid oxidative stress (Cui et al., 2004). 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 (Park et al., 2001, Giguere et al., 2003). Cd can cause oxidative stress through several mechanisms; the Fenton reaction (Stohs and Bagchi, 1995), depletion of cellular glutathione, alterations of mitochondrial electron transfer chain (Wang et al. 2004) and inhibition of antioxidant enzymes (Hansen et al., 2007). These changes may result in biochemical and morphological alterations in the affected organs.

Gestational and lactational exposure to cadmium leads to neurotoxicity and neurobehavioral changes. Such changes are observed in the developing brain in the hippocampal region associated with increased lipid peroxidation, surge in reactive oxygen species (ROS) and depressed antioxidant defense (Mukherjee et al., 2010). 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 brain (Clandinin, 1999). It could be hypothesised that the effects of Cd on the developing brain may be mediated by an effect on fatty acid composition in milk and in the brain of the suckling offspring. There are some reports showing that Cd may interact with lipid metabolism. Generally, Cd enhances lipid peroxidation in vitro and in vivo (Stohs and Bagchi, 1995). Effects on phospholipid composition have been reported in peritoneal macrophages from mice chronically exposed to Cd (Ramirez and Gimenez, 2002), in brain of pre- and postnatally Cd-exposed rats (Gulati et al., 1986; Gupta and Shukla, 1996), and in liver cells after in vitro and in vivo treatment with Cd (Kudo et al., 1990, 1991; Kudo and Waku, 1996). Liver is the main tissue where both metabolism and catabolism of fatty acids takes place. It is known that Cd exposure may lead to hepatic lipid peroxidation in rodents (Yiin et al., 2000). Several previous studies have found that Cd (Varga and Paksy, 1991) strikingly alter the testes of adult rats and mice. Oral administration of Cd to rodents results in necrosis, testicular atrophy, and sterility in males (Varga et al., 1993). Gestational exposure to cadmium induced biochemical and reproductive effects with alterations in testicular steroidogenesis and the antioxidant system of cauda-epididymis (Pillai et al., 2011). 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 previous studies concerned with two types of exposure together. A concentration of 20 ppm Cd in dam's drinking water was selected as representative of maximum levels found in food and water.

2. Materials and methods

2.1. Chemicals

Cadmium Chloride ($CdCl_2$) was purchased from Sigma Chemical Co (St. Louis, Mo., USA).). Superoxide dismutase (SOD), and lipid peroxide (MDA) were measured using commercial test kits supplied Bio-diagnostics (Bio-diagnostics, Cairo, Egypt). All other chemicals were of the highest grade available commercially.

2.2. Animals and experimental design

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), pups were removed from their mothers, sexed and randomly re-assigned to lactating females so as to provide same-sex pups per female. These lactating females received either 0 ppb (control), 20 ppm Cd as CdCl2 in their drinking water. The drinking water was administered ad libitum to female rats, as distilled water, or cadmium chloride solution prepared in distilled water, and replaced daily to minimize cadmium 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 Cadmium by the U.S. Department of Health & Human Services, to produce effects on normal development in rats.

2.3. Sample collection

On days 25 total of 30 male rat pups were weighed and euthanized by CO2 asphyxiation. The whole blood was collected 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.

2.4. 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 [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).

2.5. Determination of lipid peroxidation

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, (1978). 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.

2.6. Determination of superoxide dismutase (SOD) activity

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 (Nebot, 1991).

2.7. Determination of protein

Protein concentrations were measured by the method of Bradford (1976), 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.

2.8. Measurement of testosterone

Serum testosterone levels were measured by RIA following extraction with diethyl ether as described previously Murono et al, (1989).

2.9. Measurement of Alakaline phosphatase and Lactate dehydrogenase

Serum alkaline phosphatase and Lactate dehydrogenase (LDH) were determined colormetrically according to Vassaultt et al. (1982) and Tietz (1994), respectively.

2.10. 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).

2.11. Statistical analysis

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.

3. Results

3.1. Hematological determination

A significant ($P < 0.05$) decrease in RBC (106 /mm), Hg (g/dl), HCT (%), RDW (%), MCV(um), MCH(pg) and MCHC (g/dl) was obtained in rat pups exposed to CdCl2 through maternal milk, in comparison with control pups (Table 1). While they were significantly decreased in terms of PLT (10^3 /mm) and PDW (um) values but no significant difference was obtained in MPV (um) and PCT (%) than the control pups (Table 2).

3.2. Oxidative status in brain, liver and testes

The levels of LPO and were significantly elevated in cadmium exposed rat pups brain, liver and testes as compared to control. The cadmium-exposed rat pups showed a marked reduction ($P < 0.05$) in the activities of SOD in brain, liver and testes as compared to control (Table 3).

3.3. Biochemical parameters

A significant ($P < 0.05$) reduction in testosterone hormone concentration (g\dl) was obtained in the serum of exposed rate pups than control pups. A significant ($P < 0.05$) elevation in ALP and LDH concentration was recorded in the serum of exposed rate pups than the control (Table 4).

3.4. Histopathology

3.4.1. Brain

The brain of control, untreated rat pups, showed normal neurons of the cerebral cortex (Fig. 1a). Meanwhile, the brain of rat pups exposed to cadmium through lactation was 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).

3.4.2. 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).

3.4.3. Testes

In the testes, cadmium 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 oedema in the seminiferous tubules and interstitial tissue (Fig. 3b).

Table 1
The effect of lactational exposure to cadmium chloride on the different hematological indices in rat pups.

	RBCs 106/ml	Hg g/dl	HCT %	RDW %	MCV um	MCH pg	MCHC g/dl
Pups	7.34±0.37*	13.99±0.7*	38.76±4.2*	26.86±0.3*	46.34±2.1*	18.20±0.4*	33.14±0.3*
Control	8.74±0.31	15.00±0.5	49.28±0.6	24.45±0.4	48.72±2.5	22.88±1.7	37.40±2.0

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

Table 2
The effect of the lactational exposure to cadmium chloride on thrombocytic indices in rat pups.

	PLT 10^3 /ml	MPV um	PDW um	PCT %	LPCR %
Pups	710.4±91.3*	6.20±0.22	8.95±0.39*	0.42±0.04	6.72±1.1
Control	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 thirty rat pups per group.*denotes $P < 0.05$ as compared to control group (One- way ANOVA/Duncan).

Table 3
The effect of the lactational exposure to cadmium chloride on the oxidative status in rat pups.

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

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

4. Discussion

The toxic effects of Cd on adult rat are well documented, whereas only a few studies with administration of Cd in lactational exposure alone have been conducted in newborn rats. The results obtained in this study showed that the newborn rats exposed to Cd through maternal milk had a distortion of the hemopoietic features in the form of decrease of RBCs 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 cadmium-induced toxic effects on hematological indices of albino rats (Frank et al., 2010). Gestational and lactational exposure to Cd induce metabolic changes in the foetus, resulting in reduced haematocrit values (Prigge, 1978) and inhibition of zinc dependent enzymes (Samarawickrama and Webb, 1981). It is well known that the presence of Cd in the organism decreases the level of iron in the blood (Kostić et al., 1993) and causes the decrease of Hb concentration. The decrease of Ht value in hemolyzed plasma of rats exposed to Cd indicates the increased destruction of erythrocytes (Shukla et al*.,* 1996; Hamada et al., 1998). Moreover, cadmium may inhibit heme synthesis by decreasing the absorption of iron from the gastrointestinal tract (ATSDR, 1999).

Cadmium 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 (Manca et al., 1991). In the present study, the brain, liver and testes of neonatal rat exposed to Cd by lactation indicate the presence of oxidative stress which evidenced by the elevation in lipid peroxidation (MDA) and depressed in the antioxidant enzymes (SOD) compared to those of controls. In general, mechanisms by which Cd can induce oxidative stress through free radicals over production and the disruption of the mitochondrial membrane which appear to be the primary target to its cellular effect (Thompson and Bannigan, 2008).

Histopathological examination of brain from neonatal rats exposed to Cd revealed massive damage in the hippocampus and cerebral cortex in the form of cellular atrophy, shrinkage, cellular necrosis, cerebral hemorrhage and cerebral edema. Neurochemical disturbances of the serotonergic system in the offspring during the lactational period have been shown in rats exposed to low levels of cadmium in the drinking water (Andersson et al., 1997). In neuronal cells, cadmium induces oxidative stress, which produces protein damage (Figueiredo- Pereira et al., 1998) and subsequently neurodegeneration (Williams, 1995; Okuda et al., 1997). These changes may be attributed to the fact of cadmium is neurotoxic metal which induces cellular damage and oxidative stress through free radicals over production in brain (Armenta et al. 2003). These studies are in harmony with our results of oxidative damage which obtained in the brain tissue as mentioned before. Moreover, Mukherjee et al. (2010) documented that dietary exposure to cadmium, even at lower doses, can lead to free radical induced neurotoxicity, neurobehavioral changes and alteration in neurotransmitters. Such changes are likely to be more pronounced in the developing brain due to incompleteness of blood brain barrier.

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

	ALP **U\l**	**LDH** mM/L	**Testosterone** ng/L
Pups	85.12±10*	0.36 ±0.02*	2.06±0.22*
Control	50.43±3	0.98 ±0.14	3.24 ± 0.30

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

In the present study, mononuclear cell infiltrations and necrosis of hepatocytes were evident in the livers of neonatal rats. Moreover, a significant elevation in ALP and LDH concentration was recorded in the serum of rate pups on lactational exposure to Cd. The cadmium-induced LDH release suggests a necrotic process (Bucio et al., 1995). Furthermore, cadmium-induced necrosis accompanied by LDH release has been found in several cell types (Koizumi et al., 1996; Zimmerhackl et al., 1998). Generally, the liver is one of the critical target organs after acute and chronic exposures to cadmium (Guilhermino et al., 1998). Also, cadmium can induce lipid peroxidation in tissues (Bagchi et al. 1996), which might lead to necrosis. Cadmium-induced necrosis in the liver and caused the release of abnormal quantities of alkaline phosphatase and aminotransferases enzymes into the blood (Asagba and

Eriyamremu, 2007). Free radical-induced oxidative stress causes membrane lipid peroxidation (Gotz et al., 1993) 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 (Kuester et al., 2002).

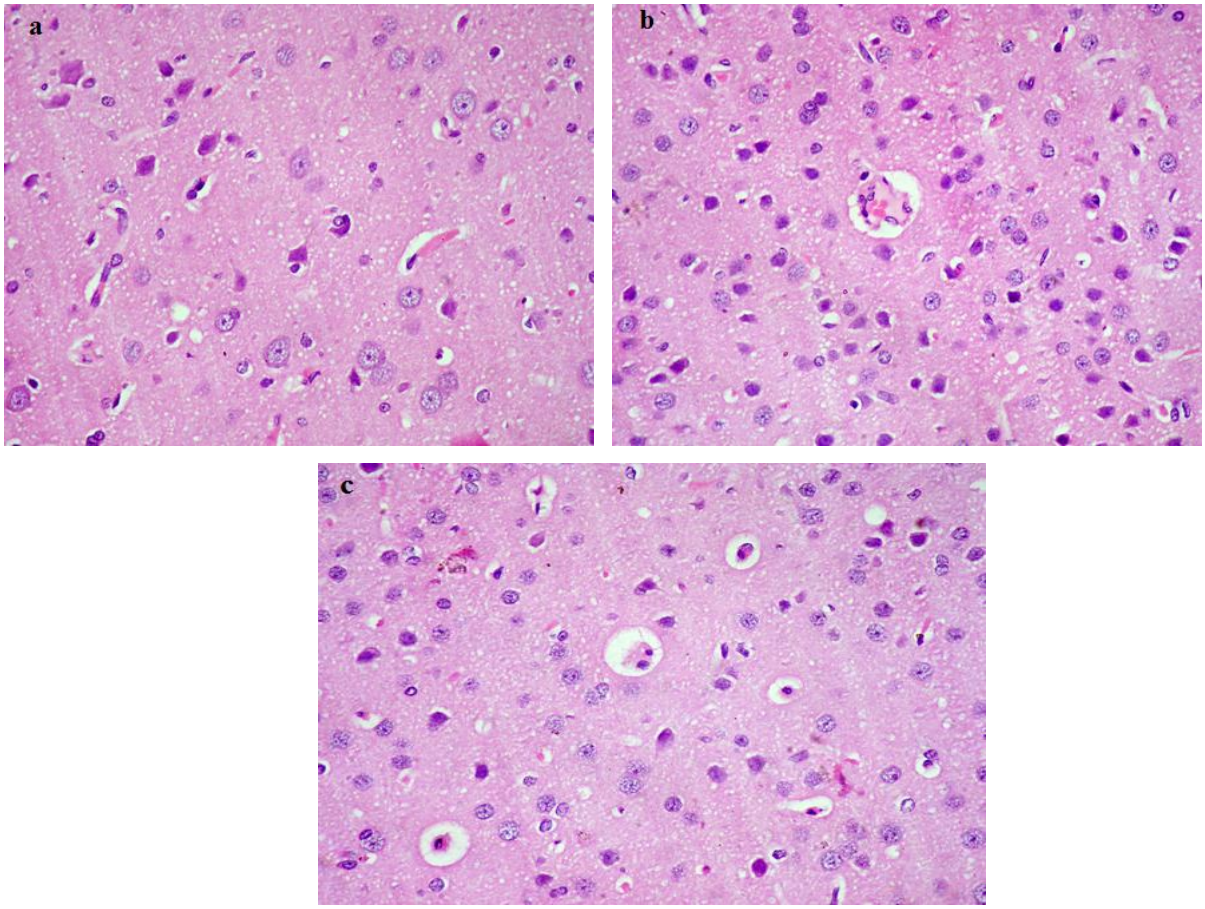

Fig. 1. Sections of the brain from control and rat pups exposed to CdCl2 through lactation, showing neurons and perineural tissues of the cerebral cortex. a) Normal neurons of the cerebral cortex in the control. H&E. X40. b) Central chromatolysis of the nucleus and cytoplasmic vaculation in the neurons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

5. Conclusion

In the current study, a significant reduction in testosterone hormone concentration in the serum of exposed rate pups was obtained along with damage to the histology of the testes. These damages were characterized by destruction of germ cells and semniferous tubules. The toxic effects of gestational and lactational exposure to cadmium on testicular steroidogenesis, antioxidant system and male accessory gland functions attributed to disturbance in the biochemical mechanisms involved in endocrine disruptions (Pillai et al., 2012). Amara et al. (2008) attributed decreased testicular growth rate and plasma testosterone to Cd-induced oxidative stress, as concurrent reduction in glutathione peroxidase, catalase, mitochondrial Mn–SOD, and cytosolic CuZn–SOD, along with increased malondialdehyde. Cd-induced oxidative stress in testicular tissues was observed in this study in the form of decrease the antioxidant enzyme SOD activity and increase in the levels of MDA lipid peroxidation product. In conclusion, lactational exposure to cd chloride can induce several alterations in new offspring as anemia liver, brain and testicular damage. Moreover, these changes mainly associated with the presence of oxidative damage indices.

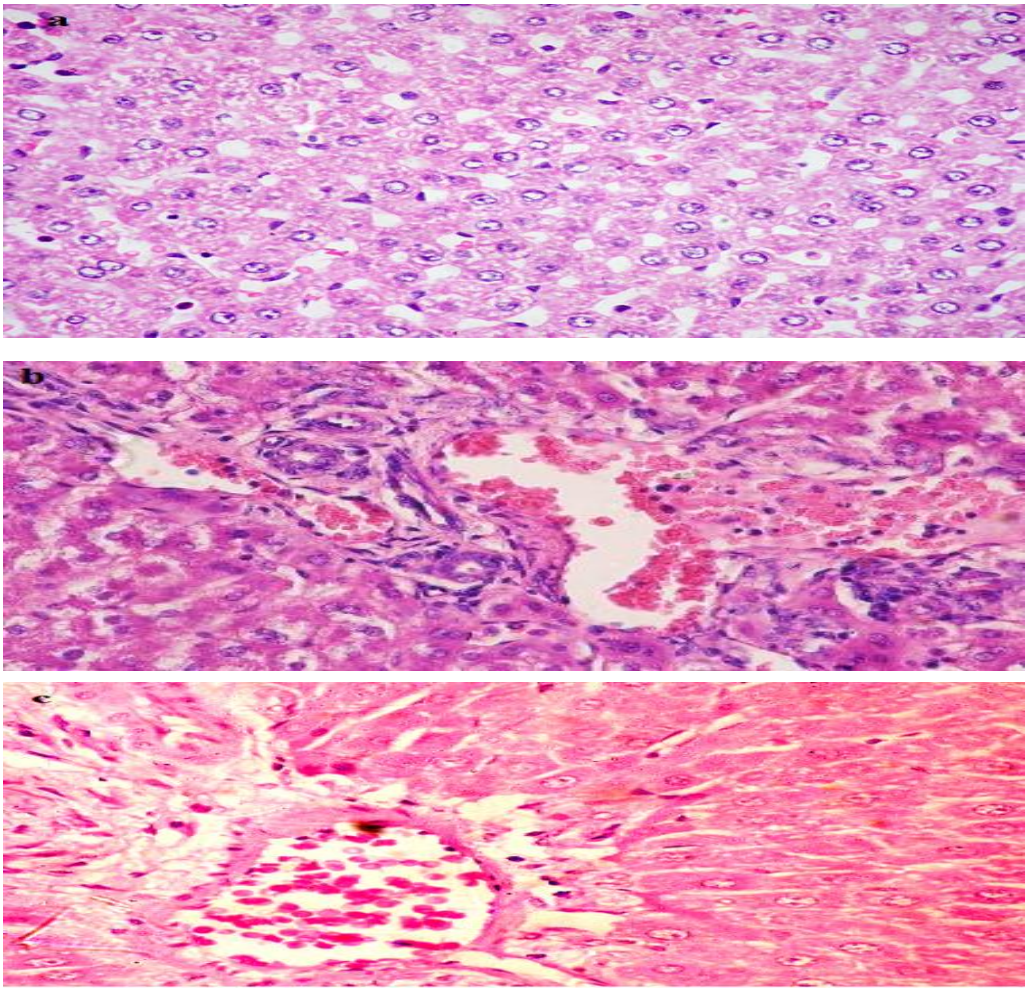

Fig. 2. Sections of the liver from rat pups exposed to CdCl2 through lactation a) Hepatic cells showed vacuolar and fatty degeneration H&E. X25. b) Congested blood vessel with cellular infiltration H&E. X40. c) The portal area showed increase in kupffer cell infiltration H&E. X40.

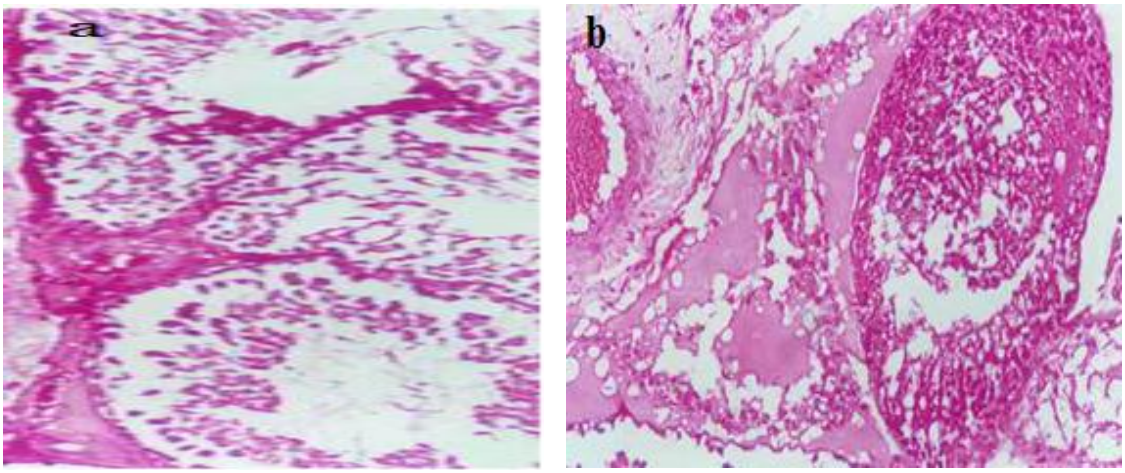

Fig. 3. Sections of the testes from rat pups exposed to CdCl2 through lactation a) Semineferous tubules showed degeneration with nuclear pyknosis in spermatocytes H&E X40. b) There is an extensive interstitial edema among the semineferous tubules H&E X40.

References

Amara, S., Abdelmelek, H., Garrel, C., Guiraud P., Douki T., 2008. Preventive effect of zinc against cadmium-induced oxidative stress in the rat testis. J. Reprod. Dev., 54: 129-134.

Andersson , H., P etersson –Grawe , K., Lindqvist , E., Luth- Man, J., O skarsson , A., Olson, L., 1997. Low-level cadmium exposure of lactating rats causes alterations in brain serotonin levels in the offspring. Neurotoxicol. Teratol., 19, 105 ±115.

Armenta, M.M., Herna´ndez, J. V., Moguel, R.B., Ruı´z, .N.C., Capdeville, M.E J., Rı´os, C, 2003. Brain regional lipid peroxidation and metallothionein levels of developing rats exposed to cadmium and dexamethasone Toxicol. Lett. 144 151_ 157.

Asagba and Eriyamremu, G. E., 2007. Oral cadmium exposure alters haematological and liver function parameters of rats fed a Nigerian-like diet J Nutr Environ Med. 16(3–4): 267–274.

ATSDR., 1999 . Toxicological profile for cadmium. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Bagchi D, Bagchi M, Hassoun EA, Stohs SJ., 1996. Cadmium induced excretion of urinary lipid metabolites, DNA damage, glutathione depletion and hepatic lipid peroxidation in Sprague–Dawley rats. Biol Trace Elem Res;52: 143–54.

Bhattacharyya, M.H., 1983. Bioavailability of orally administered cadmium and lead to the mother, fetus, and neonate during pregnancy and lactation: an overview. Sci. Total Environ. 28, 327–342.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem.7; 72:248-54

Bucio, L., Souza, V., Albores, A., Sierra , A., Chaa Vez, E., Carabez, A., Gutiea Rrez-Ruiaz, M.C., 1995. Cadmium and mercury toxicity in a human foetal hepatic cell line (WRL-68 cells). Toxicol. 102, 285 ± 299.

Buege, J.A., Aust, S.D., 1976. Lactoperoxidase catalysed lipid peroxidation of microsomal and artificial membranes. Biochem Biophys Acta; 444:192–201.

Clandinin, M.T., 1999. Brain development and assessing the supply of polyunsaturated fatty acid. Lipids 34, 131–137.

Coogan, T. P., Shiraishi, N., and Waalkes, M. P., 1994. Apparent quiescence of the metallothione in gene in rat ventral prostate: Association with cadmium-induced prostate tumors in rats. Environ. Health Perspect. 102, 137-139

Cui, K., Luo, X., Xu, K., 2004. Role of oxidative stress in neurodegeneration. Recent development in assay methods for oxidative stress and nutraceutical antioxidants. *Prog. Neuro-psychopharmacol.* Biol. Psychiatry, 28: 771-779.

Gerber, G. B., Leonard, A., Jacquet, P. 1980. Toxicity, mutagenicity and teratogenicity of lead. Mutat. Res. 76, 115-141.

Guilhermino, L., Soares, A., Carvalho AP, Lopes, M.C., 1998. Effects of cadmium and parathion exposure on hematology and blood biochemistry of adult male rats. Bull Environ Contam Toxicol; 60:52–9.

Figueiredo -Pereira, M.E., Yakushin, S., Cohen, G., 1998. Disruption of the intracellular sulfhydryl homeostasis by cadmium-induced oxidative stress leads to protein thiolation and ubiquitination in neuronal cells. J. Biol. Chem., 273, 12703 ±12709.

Frank, C., Onwuka, Osaro Erhabor,. Eteng, M.U., Umoh, I.B., 2011. Protective Effects of Ginger toward Cadmium-Induced Testes and Kidney Lipid Peroxidation and Hematological Impairment in Albino Rats. J. Med. Food. 14(7-8): 817-821.

Eklund, G., Petersson Grawé, K., Oskarsson, A., 2001. Bioavailability of cadmium from infant diets in newborn rats. Arch. Toxicol. 75, 522–530.

Eklund, G., Tallkvist, J., Oskarsson, A., 2004. A piglet model for studies of gastrointestinal uptake of cadmium in neonates. Toxicol. Lett, 146, 237–247.

Egwurugwu, J. N., Ufearo, C. S., Abanobi, O. C., Nwokocha, C. R., Duruibe, J. O., Adeleye, G. S., Ebunlomo, A. O., Odetola, A. O., Onwufuji, O., 2007. Effects of ginger (*Zingiber officinale*) on cadmium toxicity. Afr J Biotechnol Vol. 6 (18), pp. 2078-2082.

Hassan, H.M., 1997. Cytotoxicity of oxyradicals and the evolution of superoxide dismutases. In: Clerch LB, Massaro DJ, eds. Oxygen, gene expression and cellular function, (27-47). New York: Marcel.

Hamada, T., Tanimoto, A., Arima, N., Ide, Y., Sasaguri, T., S himajiri, S., M urata, Y., W ang, K.Y., S asauri, Y., 1998. Pathological study of splenomegaly associated with cadmium-induced anemia in rats. Sangyo Ika Daigaku Zasshi **20:** 11-19.

Gupta, A., Shukla, G.S., 1996. Ontogenic profile of brain lipids following perinatal exposure to Cd. J. Appl. Toxicol. 16, 227–233.

Gulati, S., Gill, K.D., Nath, R., 1986. Effect of Cd on lipid composition of the weanling rat brain. Acta. Pharmacol. Toxicol. 59, 89–93. 22.

Gotz, M.E., Kunig, G., Riederer, G.P, Youdim, M.B.H.,1994. Oxidative stress: free radical production in neural degeneration. Pharmaceut Therapeut; 63:37–122.

Giguere, A., Couillard, Y., Campbell, P., Perce, O., Hare, L., Princi-Allol, B., Pellerin, J., 2003. Steady state distribution of metals among matalothionein and other cytosolic ligands and links to cytotoxicity in bivalves living along a polymetallic gradient. Aquat. Toxicol 64, 185-200.

Katsuki, H., Okuda, S., 1995. Arachidonic acid as a neurotoxic and neurotrophic substance. Prog. Neurobiol. 46, 607–636.

Koizumi, T., S hirakura, H., Kumagai, H., T atsumoto, H., S ukuzi, K.T., 1996. Mechanism of cadmium-induced cyto-toxicity in rat hepatocytes: cadmium-induced active oxygen-related permeability changes of the plasma membrane. Toxicol, 114, 125 ± 134.

Kostic, M. M., O gnjanovic, B., D imitrijevic, S., .I kic, R.V., .T ajn, A., R osic, G.L, .Ivkovic, R.V.,: 1993 Cadmiuminduced changes of antioxidant and metabolic status in red blood cells of rats: in vivo effects. Eur J Haematol 51: 86-92.

Kuester, R.K., Waalkes, M.P., Goering, P.L., Fishers, B.L., Micuskey, R.S., Sipes, I.G., 2002. Differential hepatotoxicity induced by cadmium in Fisher 344 and Sprague–Dawley rats. Toxicol Sci; 65: 151–9.

Kudo, N., Nakagawa, Y., Waku, K., 1990. The effect of cadmium on the composition and metabolism of hepatic fatty acids in zinc-adequate and zinc-deficient rats. Toxicol. Lett. 50,203– 212.

Kudo, N., Nakagawa, Y., Waku, K., Kawashima, Y., Kozuka, H., 1991. Prevention by zinc of cadmium inhibition of stearoyl- CoA desaturase in rat liver. Toxicol. 68, 133–142.

Kudo, N., Waku, K., 1996. Cd suppresses of desaturase activity in rat hepatocytes. Toxicol. 114, 101–111.

Manca, D., Ricard, A.C., Trottier, B., Chevalier, G., 1991. Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicol. 67: 303-23.

Mukherjee, R., Desai, F., Singh, S., Gajaria, T ., Singh, P.K., Baxi, D.B., Sharma, D Bhatnagar, M ., Ramachandran, A.V., 2010. Melatonin protects against alterations in cholinergic system, trace metals and oxidative stress induced by gestational and lactational exposure to cadmium. EXCLI J *;9: 119-132*

Murono, E.P., Lin, T., Osterman, J., Nankin, H.R., 1989. The effects of cytochalasin B on testosterone synthesis by interstitial cells of rat testis. Biochem Biophys Acta;633: 228–36.

Nebot, C., Moutet, M., Huet, P., Xu, J.Z., Yadan, J.C., Chaudiere, J., 1991. Spectrophotometric Assay of Superoxide Dismutase activity based on activated autoxidation of a tetracyclic catechol. Anal. Biochem.214, 442-451.

Okuda , B., Iwamoto , Y., Tachibana, H., S ugita, M., 1997. Parkinsonism after acute cadmium poisoning. Clin. Neurol. Neurosurg., 99, 263 - 265.

Park, J.D., Y. Liu, C.D. Klaassen, 2001. Protective effect of metalothionein against the toxicity of cadmium and other metals. Toxicol. 163: 93-100.

Petersson Grawé, K., Oskarsson, A., 2000. Cadmium in milk and mammary gland in rats and mice. Arch. Toxicol. 73, 519–527.

Pillai, P., Pandya, C., Bhatt, N., Gupta, S.S., 2012. Biochemical and reproductive effects of gestational/lactational exposure to lead and cadmium with respect to testicular steroidogenesis, antioxidant system, endogenous sex steroid and cauda-epididymal functions. Andrologia. 44(2):92-101.

Prigge, E., 1978. Inhalative cadmium effects in pregnant and fetal rats. Toxicol. 10, 297–309.

Saric, M.M., Blanusa, M., Piasek, M., Varnai, V.M., Juresa, D., Kostial, K., 2002. Effect of dietary calcium on cadmium absorption and retention in suckling rats. Biometals 15, 175–182.

Serafim, A., M.J. Bebianno, 2007. Kinetic model of cadmium accumulation and elimination and metalothionein response in *Ruditapes decussates.* Environ Toxicol Chem, 26: 960-969.

Shulka , A., Shulka , G.S., Srimal, R.C., 1996. Cadmium- induced alterations in blood-brain barrier permeability and its possible correlation with decreased microvessel antioxidant potential in rat. Hum. Exp. Toxicol., 15, 400 - 405.

Sofyan, A., Rosita, G., Price, D. and Birge, W., 2007. Cadmium uptake by *Ceriodaphnia dubia* from different exposures: Relevance to body burden and toxicity. Environ. Toxicol. Chem. 26(3): 470-477.

Stohs, S.J., Bagchi, D., 1995. Oxidative mechanisms in the toxicity of metal ions. Free Radic. Biol. Med. 18, 321–336.

Tietz, N.W., 1994. Textbook of Clinical Chemistry .2nd ed. W. B. Sonuner Co.philadelphia.p.851-860.

Thawley, D.G., Willoughby, R.A., McSherry, B.J., 1977. Toxic interactions among Pb, Zn, and Cd with varying levels of dietary Ca and vitamin D: Hematological system. Environ Res 14:463-475.

Thompson, J., and Bannigan, J., 2008. Cadmium: Toxic effects on the reproductive system and the embryo. Reprod. Toxicol. 25., 304–315

Varga, B., Paksy, K., 1991. Toxic e¤ects of cadmium on LHRH induced LH release and ovulation in rats. Reprod. Toxicol. 5, 199-203.

Varga, B., Zsolnai, B., Paksy, K., Naray, M., Ungvary, G., 1993. Age dependent accumulation of cadmium in the human ovary. Reprod Toxicol; 7(3):225–8.

Ramirez, D.C., Gimenez, M.S., 2002. Lipid modification in mouse peritoneal macrophages after chronic cadmium exposure. Toxicol . 172, 1–12.

Vassaultt, *et al*, 1982. Fundamentals of Clinical Chemistry.2nd, Edn. W.B.Sandars, 478.

Wang, Y., J. Fang, S. Leonard, K. Murali, 2004. Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Radical Bio Med, 36: 1434-1443.

Williams, L.R.,1995. Oxidative stress, age-related neurodegenera- tion, and the potential for neurotrophic treatment. Cerebrovasc. Brain. Metab. Rev., 7, 55 ± 73.

Yiin, S.J., Chern, C.L., Sheu, J.Y., Lin, T.H., 2000. Cadmium induced liver, heart, and spleen lipid peroxidation in rats and protection by selenium. Biol. Trace Elem. Res. 78, 219–230.

Zimmerhackll, L.B., Momm, F., Wiegele , G., Brandis, M., 1998. Cadmium is more toxic to LLC-PK1 cells than to MDCK cells acting on the cadherin-catenin complex. Am. J. Physiol., 275, F143 ± F153.