Original Article

Toxic Effect of Cadmium Chloride Exposure on Teeth Development in Sprague Dawley Rats During Pregnancy

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ABSTRACT:

Background: Cadmium chloride ($CdCl_2$) is a widely spread environmental toxin with reported developmental toxicity. **Aim:** The aim of the present work was to investigate the developmental toxicity of $CdCl_2$ on rats' first molar histological structure, teeth mineralization through osteocalcin staining and evaluating caspase-3 expression as potential target, both intrauterine and after birth. **Methods:** Adult male and female *Sprague Dawley rats* were mated. Pregnant rats were divided into 2 groups, control group received water and experimental group received $CdCl_2$ (5mg/kg) by oral gavage from 7th to 17th day of pregnancy. Two rats from control group and 8 rats from experimental group were sacrificed at gestational day 17 (GD17). Fifteen pups were obtained in control group and 10 pups in $CdCl_2$ group. The day of parturition was deigned to be postnatal day (PND) zero (PND 0). From control group, 13 pups were sacrificed at PND10, and 13 pups at PND 20. From experimental groups, 12 pups were sacrificed at PND10, and 11 pups at PND 20. In all pups, heads were obtained for examining maxillary first molars. **Results:** Sections from the $CdCl_2$ group showed delayed development, mineralization, and eruption of first molar than the control group in GD17, PND10 and PND 20. As regard osteocalcin, the expression pattern was the same in both groups, but the intensity of the expression decreased within the $CdCl_2$ group with statistically significant difference between them. In addition, caspase-3 expression increased indicating apoptosis induction. **Conclusion**: Exposure to $CdCl_2$ during pregnancy can lead to delay in the developmental stages of the first molar teeth beside its effect on the mineralization of dentin, the delayed teeth eruption and inducing apoptosis is a possible pathway for these effects.

KEYWORDS: Cadmium chloride; Caspase-3; Developmental toxicity; Osteocalcin; Tooth development.

I. INTRODUCTION:

Tooth hard structure (enamel, dentin, and root cement) consists of calcium as the main macro-mineral with several other minerals as phosphorus and magnesium. Minerals of physiological importance and toxic metals can bioaccumulate in the calcified tissues of teeth and bone tissue (Rasmusson and Eriksson, 2001). Teeth provide an ideal biological sample for evaluating environmental exposure to toxic metal as it has limited ability to release acclimated elements into systemic fluids (Orzechowska-Wylęgała et al., 2011).

Cadmium chloride ($CdCl_2$) is widely spread, non-biodegradable with a very long biological half-life environmental pollutant. Cadmium chloride is naturally occurring, but the industrial evolution led to increase its concentration and increased risk of human exposure. Industrial sources of $CdCl_2$ include manufacturer process of alloys, highly specialized electronic product, phosphate fertilizers, and nickel-cadmium batteries. The main routes of exposure include ingestion and inhalation due to their presence in contaminated water, contaminated food (seafood, organ meats, grains, leafy vegetables, and root crops) and tobacco which is a dominant source for CdCl2 exposure (Järup and Åkesson, 2009). Cadmium chloride was listed by the World Health Organization among the top 10 chemicals of concern to human health. In addition the US Agency for Toxic Substances and Disease Registry (ATSDR) categorized CdCl2 the seventh agent on the priority list of dangerous substances (Andjelkovic et al., 2019).

One of main points of research is the effect of exposure to CdCl2 during pregnancy on normal fetal development. Previous studies reported that CdCl2 can induce recurrent abortion, alteration of Apgar 5-min score, decrease in birth weight, decrease forelimb and hindlimb bone lengths, poorer cognition, hydrocephalus, facial abnormalities, anophthalmia, and hepatic/placental cell necrosis (Aprioku et al., 2014; Jacobo-Estrada et al., 2017). Regarding the effect on teeth, although they are not the primary target for it, CdCl2 exposure was associated with increased risk of pediatric dental caries (Amr and Helal, 2010). Also $CdCl_2$ was found to affect structure and mechanics of rat incisors (Świetlicka et al., 2019).

Osteocalcin (OC; bone Gla-protein or BGP) is a major non-collagenous bone protein with high affinity for apatite crystals, is synthesized by osteoblasts and odontoblasts (Carvalho et al., 2021). Previous study reported that chronic $CdCl_2$ exposure significantly reduced serum osteocalcin level indicating significant

reduction of osteoblastic activity (Youness et al., 2012).

Caspases (cysteinyl aspartate-specific proteases) are a conserved family of enzymes that irreversibly commit cellular apoptotic death (Nicholson et al., 1995; Lavrik, 2005). All apoptotic caspases exist in normal cells as inactive enzymes analogous. When cells undergo apoptosis, these caspases become activated through one or two sequential proteolytic events that cleave the single peptide precursor into the large and small fragments that constitute the active enzyme (Thornberry and Lazebnik, 1998). Cadmium chloride induced toxicity was linked to various mechanism including cellular apoptosis. In human lymphoma cells, $CdCl_2$ has been shown to induce apoptosis by two independent pathways: the Ca2+-cal-pain and the caspase-mitochondria pathways (El Sayed et al., 2013).

From these points, the aim of the present study was to investigate the developmental toxicity of CdCl2 on rats' first molar histological structure, teeth mineralization through osteocalcin staining and evaluating caspase-3 expression as potential target, both intrauterine and after birth.

II. MATERIAL AND METHODS:

II.1. Animals:

Adult male and female *Sprague Dawley rats* (120-150gm) were purchased from Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura university. All experimental procedures were carried out in accordance with the laboratory guidelines of Ethics Committee of Faculty of Dentistry, Mansoura University, Egypt.

The parent generation of *Sprague Dawley rats* were housed under conventional standard conditions (five rats per cage at 22 ± 2°C, 60 ± 10% humidity, and a 12-h light/dark cycle, with free access to food and water). After acclimatization for a week, every female rat determined to be in oestrus or pro-oestrus of their cycle was mated with male rat in the same cage for one day. Female rats were separated after positive identification of a vaginal sperm plug by veterinary doctor using previously described method (Behringer et al., 2016), and the day was defined as designation of gestational day (gestational day) zero (GD0).

At GD0, 30 pregnant females were housed individually in plastic cages and randomly divided into two groups:

Control group: Six pregnant rats received water by oral gavage and two of them sacrificed at GD17 and 15 pups were obtained. Other four rats allowed to continue their pregnancy. The day of parturition was deigned to be postnatal day (PND) zero (PND0). Twenty-six pups, delivered from control rats, were housed, and 13 pups were

sacrificed at PND10, and 13 pups were sacrificed at PND 20.

Experimental (cadmium chloride) group: Twenty-four pregnant rats received $CdCl_2$ solution by gavage during gestation period starting from day GD7 to GD17 (time of organogenesis). $CdCl_2$ was freshly prepared daily and given at dose (5 mg/kg) corresponding 1/18 of LD50 (Tian et al., 2018). Eight rats were sacrificed at GD17, and 10 pups were obtained. Other 16 rats allowed to continue their pregnancy. The day of parturition was deigned to be PND0. Twenty-three pups were obtained from experimental rats and housed. Twelve pups were sacrificed at PND10 and 11 pups at PND 20.

II.2. Histopathological evaluation:

Pups were anesthetized with halothane and sacrificed by decapitation. The pubs' heads were rapidly excised and then fixed in 10% neutral buffered formalin for 24 hours and decalcified in 10% natural EDTA. Heads were cut in coronal direction for examination of the maxillary first molars and processed for Haematoxylin & Eosin (H&E) stain (Obi and Nwoha, 2014).

II.3. Immunohistochemistry evaluation:

The sectioned heads were immune-stained using avidin-biotin technique. The sections were stained with anti-rabbit osteocalcin and caspase-3 immune stain (Sigma- Aldrich, St Louis, USA) (Agustina et al., 2018).

Five non overlapped different fields from each slide were digitized using Olympus® digital camera installed on Olympus® microscope. The images were analysed on an Intel® Core I3® based computer using Video Test Morphology® software with a specific built-in routine for immune-stain quantification. The targeted positive immuno-stained areas were automatically selected and separated from the original image depending on the positive stain hue range, the selected area was thresholder and defined as region of interest and then a 3D histogram was constructed, and integrated density of the area was calculated.

II.4. Statistical analysis:

Data were analysed using Statistical Package for Social Science (SPSS) version 26.0. Descriptive statistics were calculated in the form of Mean ±Standard deviation (SD). Student t test was used to compare groups.

III. REULTS:

III.1. Fetal characteristics:

Food intake and body weight gain of the pregnant females in the $CdCl_2$ group were less in comparison with control group. One of the major obstacles in the present study is high rate of fetal resorption in experimental group. The fetuses of control rats were all alive, healthy, and active with average number (6-8) pups from each rat.

Regarding the experimental group, in rats sacrificed at GD17, the average number of fetuses in each rat was (1-2) and fetuses of three rats were completely resorbed. Regarding other rats, three rats did not give birth to any pups and two of them give birth to two pups which died within two days. The other 11 rats, the average number of delivered pups and continued to PND10 and PND20 from each rat was (1-2 pups). Average body weight of pups in experimental group was (4.8± 0.5) gm and in the control group (6.1 ± 0.8)gm with statistically significant difference between them (P value <0.0001).

III.2. Histopathological evaluation:

Control group: GD17 tooth germ sections showed early bell stage of the enamel organ with outer and inner enamel epithelium, stellate reticulum, and stratum intermedium cells. The dental papilla showed clear and well-defined condensation within the concavity of the enamel organ, early differentiation of odontoblast at the central cusp, the enamel organ was separated from the dental papilla with cell free zone and was still attached to the oral epithelium with the dental lamina (Figures 1- A & 1-B). Sections of PND10 showed condensed cells of the enamel organ-providing a space for the developing enamel and fully differentiated ameloblasts were observed adjacent to the enamel matrix. Fully differentiated odontoblasts with its process were observed along the pulpal wall. Dental lamina was fragmented and the connection with the oral epithelium was lost. Epithelial root sheath of Hartwig was formed and the root dentin began to deposit (Figures 1- C & 1- D). Sections of PND20 showed fully formed crown with normal dentin and pre-dentin layer, enamel was lost during decalcification process. Root formation was almost completed. The tip of the central cusp penetrated the oral mucosa and appeared in the oral cavity (Figures 1- E & 1-F).

Cadmium chloride group: GD17 tooth germ section showed early bell stage of the enamel organ like that of the control group but smaller in size and with no signs of odontoblast differentiation (Figures 2- A1 & 2-B1). Sections of PND10 showed fully differentiated ameloblasts and odontoblast, thickness of both enamel matrix and thinner dentin when compared to control group. Epithelial root sheath of Hartwig was formed but dentin deposition was limited only to the crown with no deposition on the root (Figures 2- C1 & 2- D1). Sections of PND20 showed fully formed crown with obvious interglobular dentin layer adjacent to the pre-dentin. The first molar showed delayed eruption. Although bone was resorbed, none of the cusps penetrated the oral mucosa. Root formation was also delayed in comparison to control group (Figures 2- E1 & 2-F1).

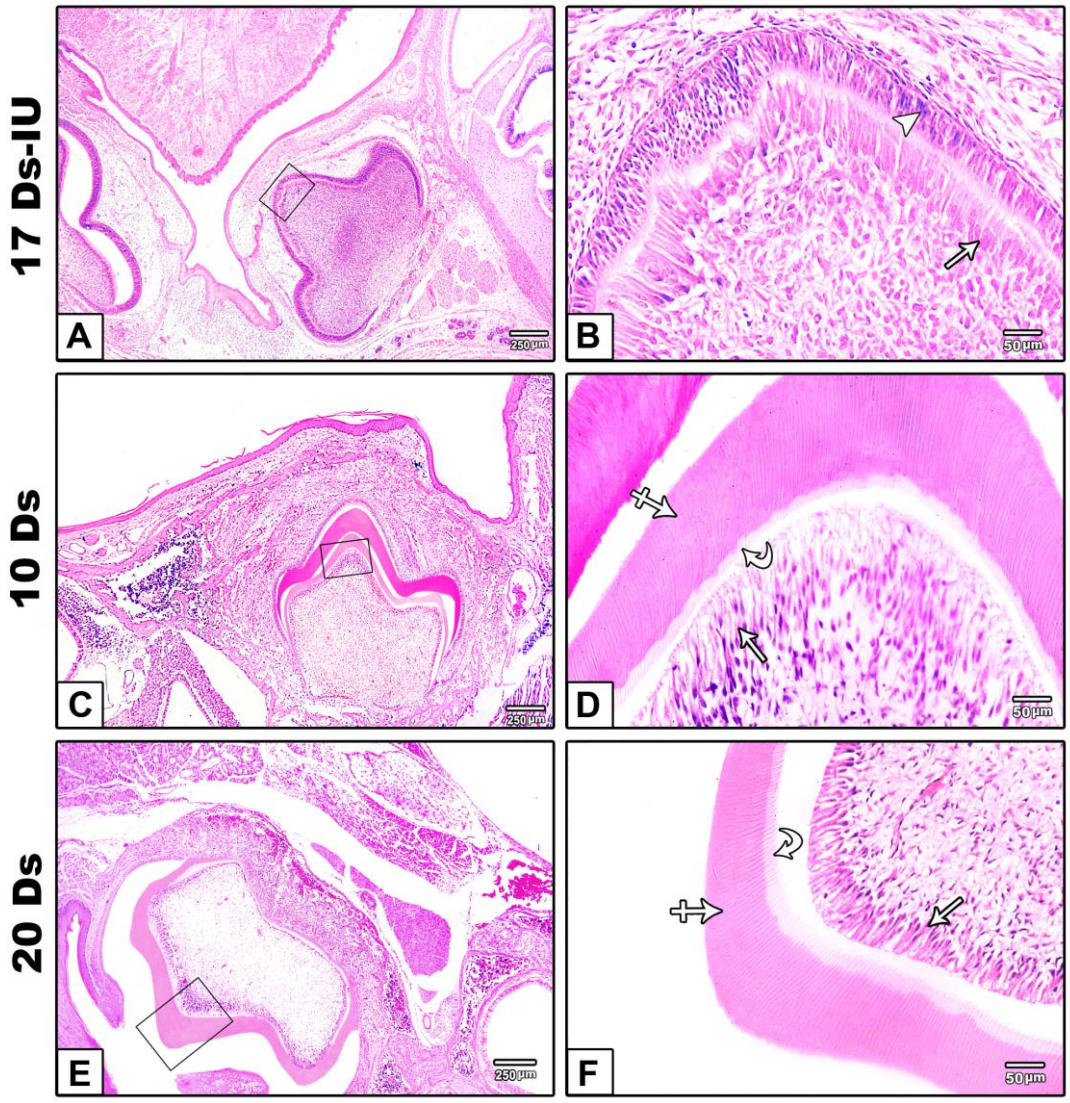

Figure 1: Photomicrographs showing a section of the developing rat molar tooth (A) section of the control group at 17th gestational day (GD17) showing normal structure of early bell stage of the enamel organ. (B) Higher magnification showing early differentiation of odontoblast at the central cusp (arrow), (arrowhead) inner dental epithelium and well defended dental papilla condensation. (C) Section of the control group at 10th postnatal day (PND10) showing fully formed layer of enamel and dentin in the crown with dentin deposition on the root. (D) Higher magnification showing fully differentiated odontoblast with its process observed along the pulpal wall (arrow) both pre-dentin (curved arrow) and dentin layer (crossed arrow). (E) Section of the control group at 20th postnatal day (PND 20) showing fully formed partially erupted crown with normal dentin and pre-dentin layer, enamel was lost during decalcification process. Root formation is almost completed. (F) Higher magnification showing full thickness of normal dentin (crossed arrow), pre-dentin (curved arrow) and odontoblasts (arrow).

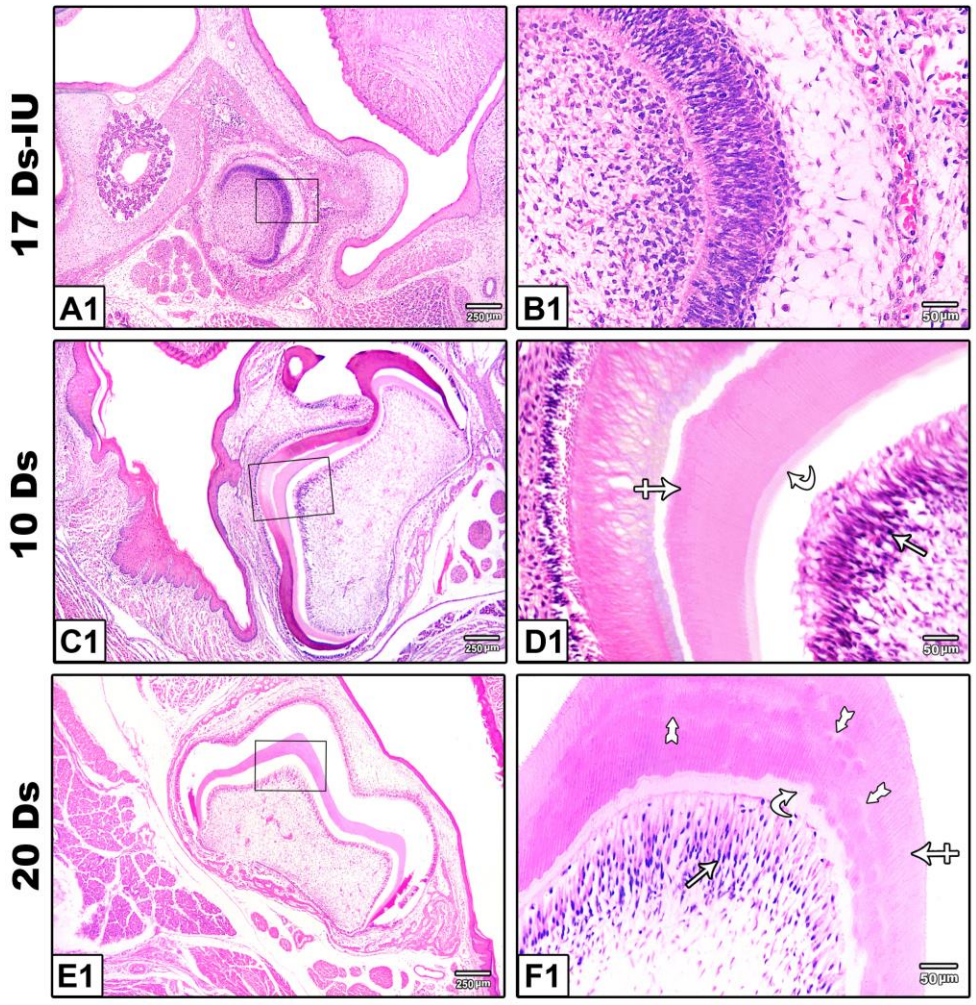

Figure 2: Photomicrographs showing a section of the developing rat molar tooth (A1) Section of the cadmium chloride ($CdCl_2$) group showing at 17th gestational day (GD17) showing smaller size early bell stage. (B1) Higher magnification of the enamel organ with no signs of odontoblast differentiation. (C1) Section of the $CdCl_2$ group at 10th postnatal day (PND10) showing fully differentiated ameloblasts and odontoblast, thinner thickness of both enamel matrix and dentin. (D1) Higher magnification showing fully differentiated odontoblast with its process observed along the pulpal wall (arrow) both pre-dentin (curved arrow) and dentin layer (crossed arrow). (E1) Section of the $CdCl_2$ group at 20th postnatal day (PND 20) showing showed fully formed unerupted crown with developing root formation. (F1) Higher magnification showing obvious interglobular dentin (tailed arrow) within the dentin (crossed arrow), pre-dentin (curved arrow) and odontoblasts (arrow). (H&E stain X 40 & X 200).

III.3. Osteocalcin staining and evaluation:

Control group: In GD17 sections, the positive reaction to osteocalcin was observed at the early differentiated odontoblast at the cusp region. In PND10 sections, odontoblast cell bodies showed positive brown reaction along with the thin layer of newly formed pre-dentin. The expression was almost the same in sections of PND20 with obvious expression of

osteocalcin_within the dentinal tubules of mature dentin (Figures 3-A, 3-C & 3-E).

Cadmium chloride group: The expression pattern was the same in both groups, but the intensity of the expression was decreased within the $CdCl_2$ group. Although the decreased positivity of odontoblasts in PND10 and PND20 but the expression in pre-dentin and mature dentin was still observed (Figures 3-B, 3-D & 3-F).

On comparing osteocalcin expression between both groups, $CdCl_2$ decreased its level in all times with statistically significant difference between $CdCl_2$ and control groups as shown in table (1).

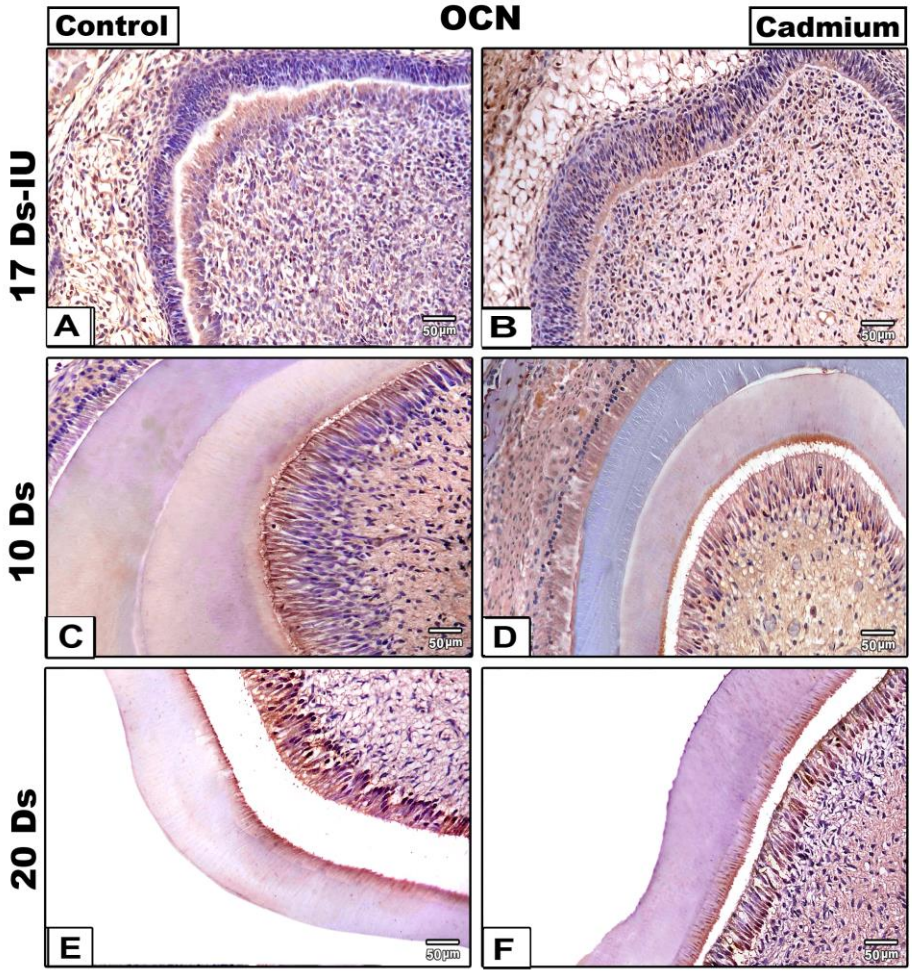

Figure 3: Photomicrographs showing a section of the developing rat molar tooth (A) Section of control group at 17th gestational day (GD17) positive cytoplasmic reaction to osteocalcin (OCN) observed in newly differentiated odontoblast at the cusp. (C) Section of control group at 10th postnatal day (PND10) showing positive reaction the cell body of odontoblasts and within the predentin layer. (E) Section of control group at 20th postnatal day (PND 20) showing more intense positive reaction of the odontoblasts, its process and the predentin layer. (B) Section of cadmium chloride ($CdCl_2$) group at GD17 showing positive reaction of the cells within the enamel organ. (D) Section of $CdCl_2$ group at PND10 showing less. positive reaction to OCN within the cell body of the odontoblasts and within the predentin layer. (F) Section of $CdCl_2$ group at PND20 showing less positive reaction to OCN in odontoblasts and dentin (Osteocalcin X 200).

Table 1: Comparison of osteocalcin expression (Area) between cadmium chloride and control groups using student t-test.

Duration of exposure	Control group		$CdCl_2$ group		p value
	Number of pups	Mean±SD	Number of pups	Mean±SD	
GD17	15	4.31±0.31	10	3.22±0.36	0.03*
PND10	13	3.31±0.34	12	2.13±0.12	0.01*
PND 20	13	1.45±0.19	11	1.05±0.03	0.01*

$CdCl_2$: cadmium chloride; GD17: 17th gestational day 17, PND10: 10th postnatal day; PND 20: 20th postnatal day. p-value is representing measured value against control group with significance level at $p < 0.05$ as significant. *: significant.

III.4. Caspase-3 staining and evaluation:

Control group: The cytoplasmic positively stained cells were observed in all time periods. The tooth germ expressed positive reaction to caspase-3 in GD17 sections. Positive reaction was observed in the cell body of odontoblast in both PND10 and PND20 sections. With the PND20 sections showed the strongest reaction (Figures 4-A, 4-C & 4-E).

Cadmium chloride group: Number of positively brown stained cells increased in all time periods relative to the control group. Odontoblasts expressing positive staining showed stronger reaction in comparison to control group in PND10 and 20 (Figures 4-B, 4-D & 4-F).

On comparing caspase-3 expression between both groups, $CdCl_2$ increased its level in all times with statistically significant difference between $CdCl_2$ and control groups as shown in table (2).

Table 2: Comparison of Caspase-3 expression between cadmium chloride and control groups using student t-test.

Duration of exposure	Control group		$CdCl_2$ group		p value
	Number of pups	Mean±SD	Number of pups	Mean±SD	
GD17	15	2.04±0.26	10	4.07±0.16	0.01*
PND10	13	1.17 ±0.43	12	1.26±0.32	0.018*
PND 20	13	1.48±0.22	11	2.52±0.24	0.02*

$CdCl_2$: cadmium chloride; GD17: 17th gestational day 17, PND10: 10th postnatal day; PND 20: 20th postnatal day. p-value is representing measured value against control group with significance level at $p < 0.05$ as significant. *: significant

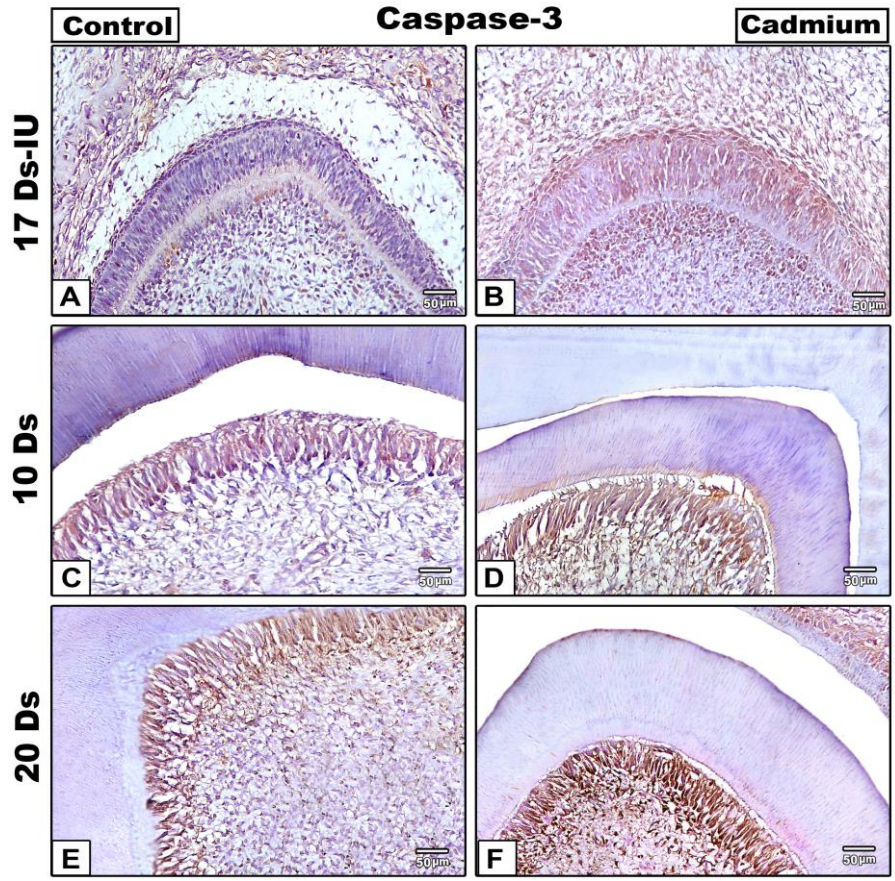

Figure 4: Photomicrographs showing a section of the developing rat molar tooth (A) Section of control group at 17th gestational day (GD17) positive cytoplasmic reaction to caspase-3 observed within the cells of the enamel organ reaction. (C) Section of control group at 10th postnatal day (PND10) showing positive cytoplasmic reaction in the cell body of odontoblast cells. (E) Section of control group at 20th postnatal day (PND 20) showing more intense positive reaction within the cell body of the odontoblast cells. (B) Section of cadmium chloride ($CdCl_2$) group at GD17 showing more positive cells to caspase 3. (D) Section of $CdCl_2$ group at PND10 showing more reaction within the cell body of odontoblast cells. (F) Section of $CdCl_2$ group at PND20 showing intense reaction within the cell body of odontoblast cells. (Caspase-3 X 200).

IV. DISCUSSION:

Cadmium chloride, widely distributed environmental toxicant, was found to induce toxic effect on teeth through affecting tooth mineral content and inducing histological and morphological changes (Ratnasari et al., 2016; Świetlicka et al., 2019). In addition, $CdCl_2$ is able to cross the placenta and produces multiple fetal anomalies (Dharmadasa et al., 2017). However, the exact effect of in utero exposure to $CdCl_2$ on tooth development either intrauterine or after birth is still not clear. From these points, the aim of the present study was to investigate the developmental toxicity of $CdCl_2$ on rats' first molar histological structure, teeth mineralization through osteocalcin staining and evaluating caspase-3 expression as potential target, both intrauterine and after birth.

In the present study, pregnant female rats showed high rate of fetal resorption and reduced number of pubs. These data are in accordance with El Sayed et al. (2013) findings on administration of CdCl2 to pregnant females. Tooth development is the result of a complex interaction between the epithelium of the first arch and the ectomesenchyme, which is derived from neural crest cells. through a series of well-defined stages epithelial thickening, bud, cap and bell the tooth is formed (Beeman and Kronmiller, 1994). Results of the control group showed early bell stage with early differentiation of odontoblasts at the cusp region at the GD17, fully differentiated odontoblasts along the pulpal wall with deposited dentin at PND10 sections and at PND20 fully formed crown with central cup penetrating the oral mucosa. These findings are in agreement with control group of previous researches (Lukinmaa et al., 2001; Silva et al., 2010; Prasanth and Saraswathi, 2012). Meanwhile, CdCl2 group data showed delayed stages in comparison with the control group in all time periods. Fetal growth retardation, a range of congenital malformation, abnormalities of embryonic development and congenital disorders of structure and function of some tissues were reported with $CdCl_2$ exposure during pregnancy and its enrich in the embryo (Salvatori et al., 2004; Ling et al., 2017). As regard defective calcification in the present results, reports of $CdCl_2$ affecting calcified tissue can contribute to explaining the observation of interglobular dentin at PND20 section indicating developmental defect in the calcified globules (Kakei et al., 2009). The possible underlying mechanism for these changes may be either indirectly through affecting the calcium and phosphorus storage in bone (Uchida et al., 2010) or directly through osteoblast dysfunction and apoptosis (Ma et al., 2021).

Odontoblasts secrete both tooth specific proteins as dentin sialoprotein and dentin phosphoprotein. Dentin is the major component of tooth hard structure with most of its protein composition is to both dentin and bone. These proteins are type I, III and V of the collagen family, osteocalcin, osteopontin, and osteonectin (Butler and Ritchie, 1995). Previous studies reported expression of osteocalcin in odontoblasts and their process within mature dentin and in pre-dentin in rats and in human (Bronckers et al., 1998; Papagerakis et al., 2002). On $CdCl_2$ exposure, odontoblasts expressed osteocalcin but at a decreased level when compared to control. Similar results were reported by Youness et al. (2012) who reported significant reduction of osteocalcin serum levels after adding cd to drinking water.

Apoptosis during tooth development has been described in rodent animal model, caspase-3 positive cells were observed in the

tooth germ in embryo day 12th to 19th (Shigemura et al., 2001). Also, the number of apoptotic cells increase but without any loss of the cell mass in postnatal morphogenesis (Matalova et al., 2004; Lungová et al., 2011). These data are in accordance with our research data for control group. The results of $CdCl_2$ group revealed increased number of positive cells to caspase-3 relative to the control. This is similar to results of previous studies on $CdCl_2$ treated rat embryos that showed increase number of apoptotic cells by confocal microscopy (Jimi et al., 2004). In addition, CdCl2 administration in mice during pregnancy caused increased primary DNA damage and activation of apoptotic pathway in their pups (Fernández et al., 2003). Apoptosis induction by CdCl2 was explained to be through inhibition of antioxidant enzymes activity (El-Boshy et al., 2015).

V. Conclusion:

In conclusion, results of the present study demonstrated the hazardous effect of exposure to $CdCl_2$ during tooth development which leads to delay in the developmental stages of the first molar teeth beside its effect on the mineralization of dentin and the delayed eruption of the teeth. In addition, increasing caspase 3 expression is possible pathway for these effects.

VI. RECOMMENDATION:

Further studies on females with high $CdCl_2$ exposure during pregnancy and the exact effect on tooth development after birth is recommended.

VII. FUNDING:

"This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors".

VIII. REFRENCES:

Agustina, H., Asyifa, I., Aziz, A., and Hernowo, B. S. (2018): The Role of Osteocalcin and Alkaline Phosphatase Immunohistochemistry in Osteosarcoma Diagnosis. *Pathology Research International*, *2018*: 1–5. https://doi.org/10.1155/2018/6346409

Amr, M. A., and Helal, A. F. I. (2010): Analysis of Trace Elements in Teeth by ICP-MS : Implications for Caries. *Journal of Physical Science*, *21*(2), 1–12.

Andjelkovic, M., Buha Djordjevic, A., Antonijevic, E., Antonijevic, B., Stanic, M., Kotur-Stevuljevic, J., Spasojevic-Kalimanovska, V., Jovanovic, M., Boricic, N., Wallace, D., and Bulat, Z. (2019): Toxic Effect of Acute Cadmium and Lead Exposure in Rat Blood, Liver, and Kidney. *International Journal of Environmental Research and Public Health*, *16*(2):

274-295. https://doi.org/10.3390/ijerph16020274

Aprioku, J. S., Ebenezer, B., and Ijomah, M. A. (2014): Toxicological Effects of Cadmium during Pregnancy in Wistar Albino Rats. Toxicology and Environmental Health Sciences, 6:16-24. https://doi.org/10.1007/s13530-014-0183-z

Beeman, C. S., and Kronmiller, J. E. (1994): Temporal distribution of endogenous retinoids in the embryonic mouse mandible. *Archives of Oral Biology*, *39*(9): 733–739. https://doi.org/10.1016/0003-9969(94)90001-9

Behringer, R., Gertsenstein, M., Nagy, K. V., and Nagy, A. (2016): Selecting Female Mice in Estrus and Checking Plugs. *Cold Spring Harbor Protocols*, *2016*(8): pdb.prot092387. https://doi.org/10.1101/pdb.prot092387

Bronckers, A. L. J. J., Price, P. A., Schrijvers, A., Bervoets, T. J. M., and Karsenty, G. (1998): Studies of osteocalcin function in dentin formation in rodent teeth. *European Journal of Oral Sciences*, *106*(3): 795–807. https://doi.org/10.1046/j.0909-8836.1998.eos106306.x

Butler, W. T., and Ritchie, H. (1995): The nature and functional significance of dentin extracellular matrix proteins. *International Journal of Developmental Biology*, *39*(1): 169–179. https://doi.org/10.1387/ijdb.7626404

Carvalho, M. S., Cabral, J. M. S., da Silva, C. L., and Vashishth, D. (2021): Bone Matrix Non-Collagenous Proteins in Tissue Engineering: Creating New Bone by Mimicking the Extracellular Matrix. *Polymers*, *13*(7): 1095. https://doi.org/10.3390/polym13071095

Dharmadasa, P., Kim, N., and Thunders, M. (2017): Maternal cadmium exposure and impact on foetal gene expression through methylation changes. *Food and Chemical Toxicology*, *109*(Pt 1): 714–720. https://doi.org/10.1016/j.fct.2017.09.002

El-Boshy, M. E., Risha, E. F., Abdelhamid, F. M., Mubarak, M. S., and Hadda, T. Ben. (2015): Protective effects of selenium against cadmium induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage in rats. *Journal of Trace Elements in Medicine and Biology*, *29*: 104–110. https://doi.org/10.1016/j.jtemb.2014.05.009

El Sayed, A., Salem, M. S., El-Garhy, A. A., Rahman, Z. A., and Kandil, A. M.

(2013): Protective effect of zinc against cadmium toxicity on pregnant rats and their fetuses at morphological, physiological and molecular level. *African Journal of Biotechnology*, *12*(16): 2110–2119. https://doi.org/10.5897/AJB12.2829

Fernández, E. L., Gustafson, A. L., Andersson, M., Hellman, B., and Dencker, L. (2003): Cadmium-induced changes in apoptotic gene expression levels and DNA damage in mouse embryos are blocked by zinc. *Toxicological Sciences*, *76*(1): 162–170. https://doi.org/10.1093/toxsci/kfg208

Jacobo-Estrada, T., Santoyo-Sánchez, M., Thévenod, F., and Barbier, O. (2017): Cadmium Handling, Toxicity and Molecular Targets Involved during Pregnancy: Lessons from Experimental Models. *International Journal of Molecular Sciences*, *18*(7): 1590-1609. https://doi.org/10.3390/ijms18071590

Järup, L., and Åkesson, A. (2009): Current status of cadmium as an environmental health problem. *Toxicology and Applied Pharmacology*, *238*(3): 201–208. https://doi.org/10.1016/j.taap.2009.04.020

Jimi, S., Uchiyama, M., Takaki, A., Suzumiya, J., and Hara, S. (2004): Mechanisms of Cell Death Induced by Cadmium and Arsenic. *Mitochondrial Pathogenesis*, *1011*: 325–331. https://doi.org/10.1007/978-3-662-41088-2_32

Kakei, M., Sakae, T., and Yoshikawa, M. (2009): Mechanism of cadmium induced crystal defects in developing rat tooth enamel. *Proceedings of the Japan Academy Series B: Physical and Biological Sciences*, *85*(10): 500–507. https://doi.org/10.2183/pjab.85.500

Lavrik, I. N. (2005): Caspases: pharmacological manipulation of cell death. *Journal of Clinical Investigation*, *115*(10): 2665–2672. https://doi.org/10.1172/JCI26252

Ling, L. Bin, Chang, Y., Liu, C. W., Lai, P. L., and Hsu, T. (2017): Oxidative stress intensity-related effects of cadmium (Cd) and paraquat (PQ) on UV-damaged-DNA binding and excision repair activities in zebrafish (Danio rerio) embryos. *Chemosphere*, *167*, 10–18. https://doi.org/10.1016/j.chemosphere.2016.09.068

Lukinmaa, P. L., Sahlberg, C., Leppäniemi, A., Partanen, A. M., Kovero, O., Pohjanvirta, R., Tuomisto, J., and Alaluusua, S. (2001): Arrest of rat molar tooth development by lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicology and Applied*

Pharmacology, *173*(1): 38–47. https://doi.org/10.1006/taap.2001.9155

Lungová, V., Radlanski, R. J., Tucker, A. S., Renz, H., Míšek, I., and Matalová, E. (2011): Tooth-bone morphogenesis during postnatal stages of mouse first molar development. *Journal of Anatomy*, *218*(6): 699–716. https://doi.org/10.1111/j.1469-7580.2011.01367.x

Ma, Y., Ran, D., Shi, X., Zhao, H., and Liu, Z. (2021): Cadmium toxicity: A role in bone cell function and teeth development. *Science of The Total Environment*, *769*: 144646-144655. https://doi.org/10.1016/j.scitotenv.2020.144646

Matalova, E., Tucker, A. S., and Sharpe, P. T. (2004): Death in the life of a tooth. *Journal of Dental Research*, *83*(1): 11–16. https://doi.org/10.1177/154405910408300103

Nicholson, D. W., Ali, A., Thornberry, N. A., Vaillancourt, J. P., Ding, C. K., Gallant, M., Gareau, Y., Griffin, P. R., Labelle, M., Lazebnik, Y. A., Munday, N. A., Raju, S. M., Smulson, M. E., Yamin, T. T., Yu, V. L., and Miller, D. K. (1995): Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature*, *376*(6535): 37–43. https://doi.org/10.1038/376037a0

Obi, A. U., and Nwoha, P. U. (2014): Effects of Kolaviron, the Major Constituent of Garcinia kola, on the Histology of the Hypothalamus, Pituitary, and Testes Using Adult Male Wistar Rats as a Model Organism. *Forensic Medicine and Anatomy Research*, *02*(03): 80–87. https://doi.org/10.4236/fmar.2014.23014

Orzechowska-Wylęgała, B., Obuchowicz, A., Malara, P., Fischer, A., and Kalita, B. (2011): Cadmium and lead accumulate in the deciduous teeth of children with celiac disease or food allergies. *International Journal of Stomatology and Occlusion Medicine*, *4*(1): 28–31. https://doi.org/10.1007/s12548-011-0005-8

Papagerakis, P., Berdal, A., Mesbah, M., Peuchmaur, M., Malaval, L., Nydegger, J., Simmer, J., and Macdougall, M. (2002): Investigation of osteocalcin, osteonectin, and dentin sialophosphoprotein in developing human teeth. *Bone*, *30*(2): 377–385. https://doi.org/10.1016/S8756-3282(01)00683-4

Prasanth, T., and Saraswathi, T. R. (2012): Histopathological and radiographic evaluation of rat molar teeth after traumatic injury-a pilot study. *Journal of Oral and Maxillofacial Pathology*, *16*(3): 313–317.

https://doi.org/10.4103/0973-029X.102473

Rasmusson, C. G., and Eriksson, M. A. (2001): Celiac disease and mineralisation disturbances of permanent teeth. *International Journal of Paediatric Dentistry*, *11*(3): 179–183. https://doi.org/10.1046/j.1365-263X.2001.00260.x

Ratnasari, D. A., Permana, M. R. S., N, Y. I., Dewi, N., and Suhartono, E. (2016): The Impact of Cadmium Exposure on Several Tooth Mineral Content. *International Journal of Pharmaceutical and Clinical Research*, *8*(8): 1240–1244.

Salvatori, F., Talassi, C. B., Salzgeber, S. A., Spinosa, H. S., and Bernardi, M. M. (2004): Embryotoxic and long-term effects of cadmium exposure during embryogenesis in rats. *Neurotoxicology and Teratology*, *26*(5): 673–680. https://doi.org/10.1016/j.ntt.2004.05.001

Shigemura, N., Kiyoshima, T., Sakai, T., Matsuo, K., Momoi, T., Yamaza, H., Kobayashi, I., Wada, H., Akamine, A., and Sakai, H. (2001): Localization of activated caspase-3-positive and apoptotic cells in the developing tooth germ of the mouse lower first molar. *Histochemical Journal*, *33*(5): 253–258. https://doi.org/10.1023/A:1017900305661

Silva, I. H. M., Leão, J. C., Evêncio, L. B., Porter, S. R., and de Castro, R. M. (2010): Morphological analysis of the enamel organ in rats treated with fluoxetine. *Clinics*, *65*(1): 61–65. https://doi.org/10.1590/S1807-59322010000100010

Świetlicka, I., Tomaszewska, E., Muszyński, S., Valverde Piedra, J. L., Świetlicki, M., Prószyński, A., Cieślak, K., Wiącek, D., Szymańczyk, S., and Kamiński, D. (2019): The effect of cadmium exposition on the structure and mechanical properties of rat incisors. *PLOS ONE*, *14*(4): e0215370. https://doi.org/10.1371/journal.pone.0215370

Thornberry, N. A., and Lazebnik, Y. (1998): Caspases: Enemies within. *Science*, *281*(5381): 1312–1316. https://doi.org/10.1126/science.281.5381.1312

Tian, H., Chen, S., Leng, Y., Li, T., Li, Z., Chen, H., and Zhang, Q. (2018): Exposure to cadmium during gestation and lactation affects development and function of Leydig cells in male offspring. *Environmental Toxicology*, *33*(3): 351–360. https://doi.org/10.1002/tox.22522

Uchida, H., Kurata, Y., Hiratsuka, H., and Umemura, T. (2010): The effects of a vitamin D - Deficient diet on chronic

cadmium exposure in rats. *Toxicologic Pathology*, *38*(5): 730–737. https://doi.org/10.1177/0192623310374328

Youness, E. R., Mohammed, N. A., and Morsy, F. A. (2012): Cadmium impact and osteoporosis: Mechanism of action. *Toxicology Mechanisms and Methods*, *22*(7): 560–567. https://doi.org/10.3109/15376516.2012.702796

الملخص العربي

التأثير السمي الناتج من التعرض للكادميوم خلال الحمل على نمو أسنان جرذان سبراغ داولي

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مقدمة البحث: الكادميوم هو أحد السموم البيئية واسعة الانتشار وله العديد من الآثار الجانبية المعروفة. **الهدف من البحث:** الهدف من البحث هو دراسة تأثير التعرض لكلوريد الكادميوم في الجرذان خلال فترة الحمل على التركيب النسيجي للطاحن الأول من أسنان صغار الجرذان وتمعدنها وتقييم تأثيره على نشاط كاسبيز-٣. **طريقة البحث:** تم تزاوج ذكور وإناث جرذان سبراغ داولي البالغة ثم قسمت إناث الجرذان الحوامل إلى مجموعتين: المجموعة الضابطة حيث تم إعطاء الجرذان الماء فقط بينما تم إعطاء كلوريد الكادميوم بجرعة ٥ملجم / كجم للمجموعة الأخرى عن طريق الفم من اليوم السابع إلى السابع عشر من الحمل. في كلا المجموعتين، تم ذبح بعض الجرذان في اليوم السابع عشر من الحمل للحصول على صغارها في حين ان باقي الجرذان أكملت الحمل لتضع صغارها. تم رعاية صغار الجرذان وتم تقسيمها ليتم ذبح ١٢ جرذ في اليوم العاشر بعد الولادة وذبح ١١ جرذ في اليوم العشرين بعد الولادة وذلك في مجموعتي الدراسة. في جميع العينات تم أخذ الرأس لفحصها. **النتائج:** أظهر فحص مقاطع من فك صغار الجرذان تأخر في تكوين، تمعدن وظهور الطاحن الأول في جميع مراحل الدراسة عن المجموعة الضابطة. بالنسبة لتكوين الاستيوكالسين فنمط تكوينه متماثل في المجموعتين، ولكن معدل تكوينه اقل في مجموعة كلوريد الكادميوم عن المجموعة الضابطة. بالإضافة لذلك كان معدل نشاط الكاسبيز-٣ زائد في مجموعة الدراسة عن المجموعة الضابطة مما يشير ان التأثير السمي لكلوريد الكادميوم يتم عن طريق زيادة الموت المبرمج للخلايا. **الخلاصة:** نستخلص من هذه الدراسة أن التعرض لكلوريد الكادميوم أثناء الحمل يمكن أن يؤدي إلى تأخير مراحل نمو الطاحن الأول إلى جانب تأثيره على تمعدن الأسنان، وتأخر ظهورها، وتحفيز موت الخلايا المبرمج هو مسار محتمل لهذه التأثيرات. لذلك يوصي بعدم التعرض لاي من مصادر الكادميوم خلال فترة الحمل لما له من تأثير سلبي على نمو الأجنة بصفة عامة وضهور وتمعدن الأسنان بصفة خاصة.