InVet. 2012, 14(2): 209-220
ISSN 1514-6634 (impreso)
ISSN 1668-3498 (en línea)

Efecto del cadmio en órganos maternos de ratas y fetos en diferentes tiempos de gestación

Effect of cadmium on maternal organs of rats and fetuses at different times of gestation

DÍAZ, M.[1]; QUIROGA, M.A.[2]; LANDA, R.[2]; GIORDANO, A.[2]; NAJLE, R.[1]

[1]Departamento de Ciencias Biológicas, [2]Centro de Investigación Veterinaria de Tandil (CIVETAN) CONICET, Departamento de Fisiopatología. Facultad de Ciencias Veterinarias; Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Campus Universitario; Paraje Arroyo Seco s/n (7000) Tandil, ARGENTINA. Fax 54 0249 4439850

RESUMEN

Para evaluar los efectos de la administración de cadmio (Cd) a diferentes tiempos de gestación, 24 ratas fueron preñadas bajo condiciones estandarizadas de crianza. Confirmada la preñez, las ratas fueron asignadas al azar a 4 tratamientos, que recibieron una dosis subcutánea de 10 mg Cd por kg PV a los días 7(GI); 9(GII) y 11(GIII) de preñez; o una dosis equivalente de solución fisiológica (GIV – control). El día 20 posconcepción, todas las ratas fueron sacrificadas. Se obtuvieron muestras de hígado, riñón, bazo, pulmón, fetos y placenta, para determinar la concentración tisular de Cd y para estudios histológicos. Los resultados fueron analizados estadísticamente (ANOVA y test t de Student). En los órganos de ratas de los grupos GI, GII and GIII, las concentraciones de Cd fueron significativamente más elevadas que en el control ($p<0.05$). En los grupos tratados con Cd, se observaron riñones con núcleos picnóticos en los túbulos de la corteza renal; hígado con infiltrado leucocítico multifocal, vacuolización celular en la zona centrolobulillar y cuerpos apoptóticos. En las placentas, se determinaron núcleos picnóticos, depósitos fibrinoides e infiltración de granulocitos. Se observó agenesia de extremidades, cola y cráneo en los fetos.

Palabras clave: (ratas), (cadmio toxicidad), (órganos maternos), (preñez), (fetos).

Correspondencia *e-mail*: Maria del Carmen Diaz maridel@vet.unicen.edu.ar
Recibido: 10-07-2012
Aceptado: 06-07-2013

SUMMARY

To evaluate the effects of the cadmium (Cd) administration at different gestation times, 24 rats were mated under standard rearing conditions. After pregnancy confirmation, rats were randomly assigned to 4 treatment groups. Experimental groups received a subcutaneous dose of 10 mg Cd per kg of body weight at the day: 7 Group I(GI); 9 Group II(GII) and 11 Group III(GIII) of pregnancy. The control group (Group IV- GIV) received an equivalent volume of saline solution. On day 20 post-conception, all rats were sacrificed. Samples of liver, kidney, spleen, lung, placenta and fetuses were collected to determine Cd concentration and for histological studies. The results were analyzed statistically (ANOVA and Student's t test). In the organs and fetuses of rats from GI; GII and GIII, Cd concentrations were significantly higher than in the control (P<0.05). In Cd-treated groups, were observed: kidneys with picnotic nuclei in tubules of the renal cortex; liver with multifocal leukocytic infiltrate, cellular vacuolization in the centrolobular zone; lungs with atelectasis and alveolar emphysema; placentas with picnotic nuclei, fibrinoid deposits and infiltration of granulocytes. Bone lesions were observed in fetuses, such as limb, tail and skull bones agenesis.

Key words: (rats), (cadmium toxicity), (maternal organs), (pregnancy), (fetuses).

INTRODUCTION

The exposition to heavy metals generates deleterious effects on the reproduction and prenatal development of man and animals. Among those metals considered as toxicant is cadmium (Cd) an element considered as non-essential for animal physiology.

Cadmium is widely distributed in the environment (air, soil, water)[31]. It is not biodegradable and is present in the nature forming different oxides and salts (carbonates, sulphates, sulphites or chlorides)[19].

Modern agricultural and industrial practices have favored the exposure to Cd and other metals, since these elements are frequently found in insecticides, fungicides, phosphated fertilizers, paints, and batteries[18, 23]. Cadmium can also contaminate the air during the meeting process of rocks to extracting Zn, Cu, and Pb[31].

Cadmium can cause damage to different tissues of many organs in mammals (kidney, liver, pancreas, ovaries, testicles, placenta and bones, among others)[1, 20, 22, 25, 42].

The lung is a target organ for cadmium toxicity[13, 38]. It has been reported that exposure to cadmium chloride by inhalation induced lesions in the alveolar septum in rats[11].

Data showed that Cd produced enhanced lipid peroxidation in the liver, heart and spleen[32]. Among the Cd teratogenic effects, it is highlighted the embryologic and fetal toxicities[26]. Studies performed in rats with ^{109}Cd corroborated that Cd cross the placenta and accumulate in fetuses[29]. Embryos can be affected depending on the stage of development and time of exposure. In newborns, Cd toxicity seems to be dependant on other factors, such as the development to the hemato-encephalic barrier.

Among other possible sources of pollution, it can be cited contaminated food products such as vegetables, edible tissues of cattle (liver, kidney), fishes, seafood and mollusks[20].

According to studies performed by Kjëllstrom[25] alterations determined by intoxication with Cd are very variables, determining acute or chronic effects that depend on factors such as the dose, time of exposition and the route of contamination[38].

Both direct and indirect exposition to Cd can be deleterious to human health. The effects of an indirect exposition can be also observed in the progeny. The Cd exposition previous to pregnancy or during it can affect the body weight of the offspring[41].

The mechanism of action of Cd on the fetus has not been elucidated yet. Proposed mechanisms are changes of metabolic processes in the maternal organism or disturbances in trophic functions of the placenta[10].

The placenta of rats is similar to that of the woman, and it has been used as an animal model for studies of teratogenicity induced by Cd[4, 10, 14, 41].

The objective of the present work was to evaluate the effects of Cd administration at different times of pregnancy, by reporting on Cd tissue concentrations and morphology of maternal organs, placenta and fetuses.

MATERIALS AND METHODS

Animals

Twenty four (24) virgin female *Wistar* rats, 4 month-old and weighing 242.8 ± 22.3g (Laboratory Animal Rearing Facilities, Faculty of Veterinary Science, Universidad Nacional del Centro de la Provincia de Buenos Aires)[5]. Animals were housed in 12 plastic boxes in standard rearing conditions (temperature 22º ± 2ºC, relative humidity 50-60%, and a light period of 12 hours). Extruded feed and water *ad libitum* were provided during the experimental period.

Reproductive program

Rats were mated with 6-month-old males in a female: male rate of 2:1. Pregnancy was confirmed by the presence of spermatozoa in the vaginal fluid (day 0). Pregnant females were single housed and weighed on days 0 and 20 of pregnancy to determining the gestation status.

Treatments

Pregnant rats were assigned to 4 groups. Three groups received a subcutaneous dose equivalent to 10 mg of Cd^{+2} (as $CdCl_2.H_2O$) per kg of BW. The times of administration for the experimental groups were at the following pregnancy days: 7, Group I (GI); 9, Group II (GII); and 11, Group III (GIII). The fourth group was the control group, Group IV (GIV), which was administered an equal volume of saline solution.

Sample collection

On day 20 post-conception, all the gestating rats were sacrificed according to the methods established by the Animal Welfare Act of the Faculty of Veterinary Science[22]. In this particular case, an overdose of ethylic ether was used.

Samples of the following maternal organs were collected: liver, kidney, spleen, lung and placenta. A portion of the sampled organs was dried to constant weight to determining the Cd concentration by atomic absorption spectrophotometry (AAS).

Some of the analyzed organs (liver, kidney and placenta) were fixed with buffered formalin at a 10% concentration in PBS, for further histological studies.

Morphological determinations. Weight of each litter was determined, as well as the following lengths: total, head to tailhead (HTL), and head (HL). Placentas were weighed and diameters measured.

Determination of Cd concentration. Samples of maternal organs (liver, kidney, spleen and lung) placentas and fetuses were dried at 70ºC until a constant weight was achieved.

Approximately 50 mg of the samples were homogenized and digested with 0.5 mL of nitric acid 70% during 6 hours at room temperature and for 12 hours at 90ºC. Cd concentrations were determined by AAS, by using a GBC 906 equipment.

Histological examination. Samples of kidney, liver, lung, spleen, placenta and fetuses were fixed in 10% neutral buffered formalin, dehydrated with ethanol and xylene, embedded in paraffin, cutted into 5 µm slices and stained with Hematoxylin and Eosin for microscopic examination.

Samples of fetuses were fixed in ethanol 96º and their organs were removed. The skeletons of fetuses were examined by the Dawson alizarin-red staining technique (Dawson technique modified by Barrow)[7].

Statistical analysis. Results of morphological measures and Cd tissue concentrations in the different organs of each group were analyzed by ANOVA and Student's t test. Differences

with a $p < 0.05$ were considered as statistically significant and were included in tables that were constructed depicting the main values and the standard deviations for each experimental group.

RESULTS

Morphological determinations

Weights and diameters of placentas from Cd-treated (groups I, II and III) and control animals (group IV) were similars; observed differences were statistically non-significant ($p>0.05$). (Table 1).

The size of the litter was lower only in G II compared to the control group.

The mean gestational sac weight and the mean fetal weight were lower and the mean length, HTL and HL were shorter in treated animals than in the control group. However, differences were statistically non-significant ($p>0.05$) (Table 2).

Cadmium tissue concentrations

Cadmium concentrations (mean ± SD), expressed as ppm DM in the tissues of the analyzed organs are presented in Table 3. In the organs of rats treated with Cd (GI; GII and GIII), significantly higher concentrations compared to those in the control group were observed ($p<0.05$). Cd concentrations differed between the analyzed organs, being in descendant order: *kidney* > *liver* > *spleen* > *lung* > *placenta* > *fetuses.*

Cd levels were higher in the different organs of G III, evidencing an inverse relation between Cd concentration and time from treatment to sacrifice. However, in the spleen, that tendency was not observed.

Histological examination

The main observations performed in the slices of kidneys, liver, lungs, spleen, fetuses and placenta belonging to animals of the different

Table 1. Placental morphometric measures (mean ± S.E) of rats sacrificed on day 20 of pregnancy from different experimental groups (n=6).

Parameter	Control (G IV) (6)	Group I (6)	Group II (6)	Group III (6)
Weight of placenta (g)	0.62 ± 0.02	0.59 ± 0.02	0.57 ± 0.02	0.58 ± 0.02
Diameter of placenta (mm)	1.33 ± 0.02	1.34 ± 0.02	1.28 ± 0.02	1.26 ± 0.02

Statistically non-significant differences were observed between treated groups versus control ($p>0.05$).

Table 2. Fetal morphometric measures (mean ± S.E.) of rats sacrificed on day 20 of pregnancy from different experimental groups (n=6).

Parameter	Control (G IV) (6)	Group I (6)	Group II (6)	Group III (6)
Litter	10 ± 1.22	11 ± 1.82	8 ± 1.72	11 ± 3.60
Weight of gestacional sac (g)	4.92 ± 0.11	4.47 ± 0.10	3.86 ± 0.11	4.27 ± 0.13
Foetus weight (g)	3.95 ± 0.11	3.47 ± 0.09	2.94 ± 0.09	3,39 ± 0.12
Foetus length (cm)	3.55 ± 0.04	3.30 ± 0.04	3.06 ± 0.05	3.23 ± 0.06
HTL (cm)	2.81 ± 0.04	2.63 ± 0.03	2.42 ± 0.04	2.57 ± 0.05
HL (cm)	1.40 ± 0.02	1.38 ± 0.02	1.33 ± 0.02	1.36 ± 0.02

Litter: number of fetuses by rat; HTL: Head to Tail Length; HL: Head Length.

Statistically non-significant differences were observed between treated groups versus control ($p>0.05$).

Table 3. Cadmium concentration (ppm DM) (Mean ± DS) determined in the organs of rats, placenta and fetuses in the different experimental groups (n=6).

	Kidneys	Liver	Spleen	Lungs	Placenta	Fetuses	Rate Pl/F
Group	(Mean ± SD)						
Control (G IV)	5.47 ± 3.29	10.56 ± 8.41	1.37 ± 1.29	0.78 ± 0.33	0.77 ± 0.27	1,05 ± 0,33	0,73 : 1
G I	321.02 (a) ± 200.71	252.15 (a) ± 100.85	41.37 (a) ± 23.57	11.75 (a) ± 8.12	9.12 (a) ± 8.42	2,96 (a) ± 1,82	3,34 : 1
G II	322.77 (a) ± 163.10	254.11 (a) ± 117.75	40.07 (a) ± 23.38	13.45 (a) ± 7.63	9.89 (a) ± 7.05	2,32 (a) ± 1,22	3,93 : 1
G III	361.42 (a) ± 130.58	326.93 (a) ± 160.99	23.07 (a) ± 18.24	18.10 (a) ± 10.71	19.95 (a) ± 11.38	6,43 (a) ± 5,91	3,10 : 1

Rate Pl/ F: Cd concentrations relationship between placenta vs. fetuses.
Differences between treated and control group are statistically significant at $p < 0.05$ (a).

experimental groups are described:

Kidneys. In all the three Cd treated groups, tubular cells presented picnotic nuclei (Figure 1)

In G II, tubules in the renal cortex showed a turbid tumefaction.

Liver. It was observed in Group I, multifocal leukocytic infiltrate, loss of cellular details, cellular vacuolization in the centrolobular zone (Figure 2); in other groups, congestion area, with multiple focuses of necrosis and leukocytic infiltrate.

Lungs. It was observed emphysema, atelectasis and congestion (Figure 3), epithelial desquamation of bronchioles in all treated groups and fibrinoid deposits in G III.

Spleen. Disorganization of the white pulp and hemosiderine deposits were observed in animals of groups I and II.

Placenta. It showed trophoblast necrosis in G III (Figure 4), hemorrhage and picnotic nuclei of giant cells in the placenta of Group I.

Fetuses. Some fetuses of the GI, GII and GIII presented incomplete ossification of the vertebrae , while other showed alterations of the architecture of the vertebral pericondrium and limbs (Figure 5, b1 y b2).

Using the Alizarin red technique it was observed lack of head bones and incomplete ossification of the vertebrae in some fetuses of G III. Also, some fetuses of GI, G II and G III presented bone alterations in the limbs (Figure 6).

DISCUSSION

Cadmium is one of the heavy metals that can affect most of the organs, apparatus and systems of the mammal body[20, 31].

Liver, kidney, lung and bones are the most affected organs[9, 33].

The severity of these alterations depends, among other factors, upon the exposition route, dose, and time of exposition to the pollutant[38, 46].

In gestating women exposed to high doses of Cd, placental and fetal alterations may occur.

Different administration routes have been proposed to perform intoxication experiences. Among them are the oral and the subcutaneous routes[2].

The percentages of Cd absorbed differ depending on the route of exposition[31]. When the subcutaneous route is used, higher organic concentrations are achieved compared to those alter the oral administration. This can be explained because in the subcutaneous route there is no competition for absorption with other elements as could be expected at the intestinal cell lining[46].

There is some controversy in determining the lethal dose, the LD50 and the minimum toxic dose for Cd[30]. As a result of the bibliographic review, we decided to administer a dose equivalent to 10 mg/kg of body weight, which is similar to the 8 mg/kg dose used by Zhao *et*

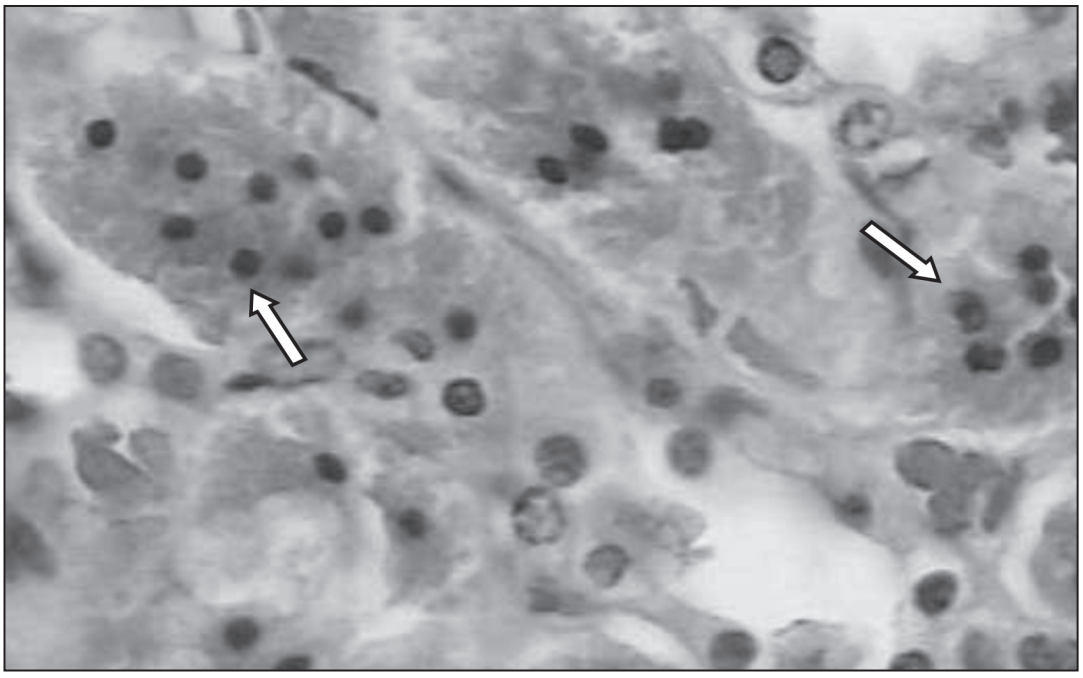

Figure 1. Renal cortex from Group II rats (day 9). Arrows indicate picnotic nuclei (necrosis) of the tubular cells. (Hematoxyline-Eosin, (H&E) 40x)

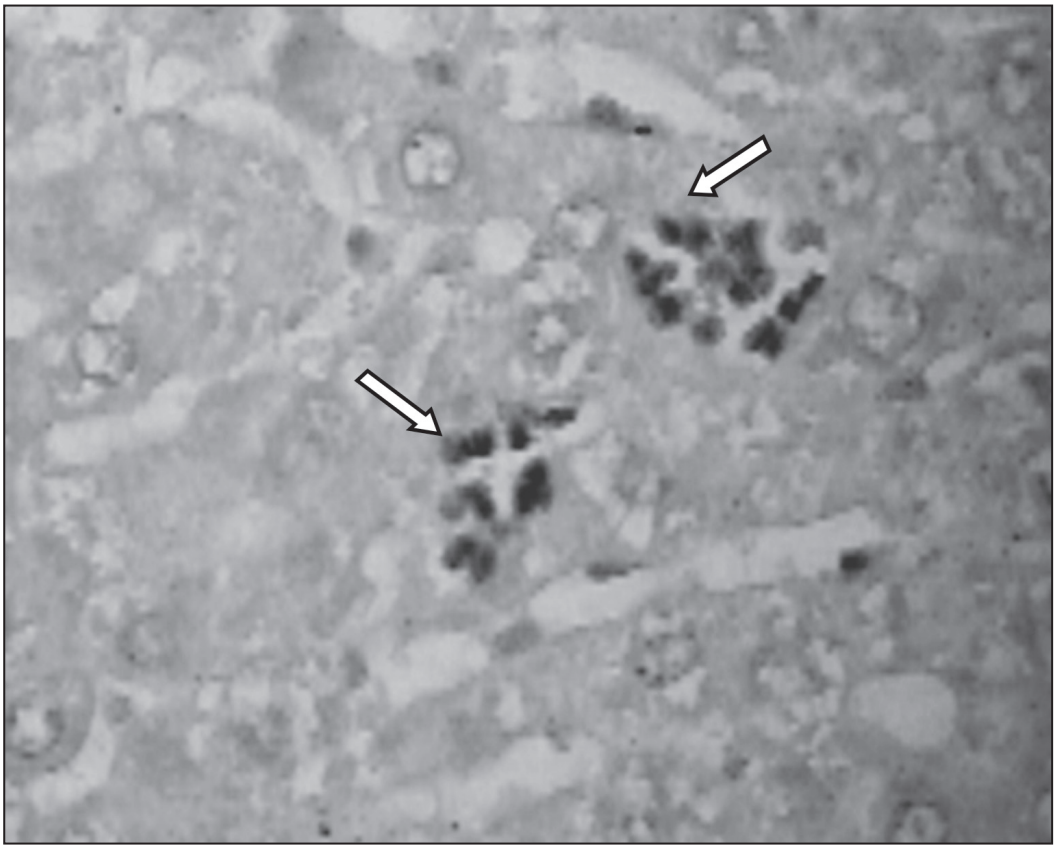

Figure 2. Liver slice from Group I rats (day 7). Arrows indicate the leukocytes infiltrate. (H &E, 40x)

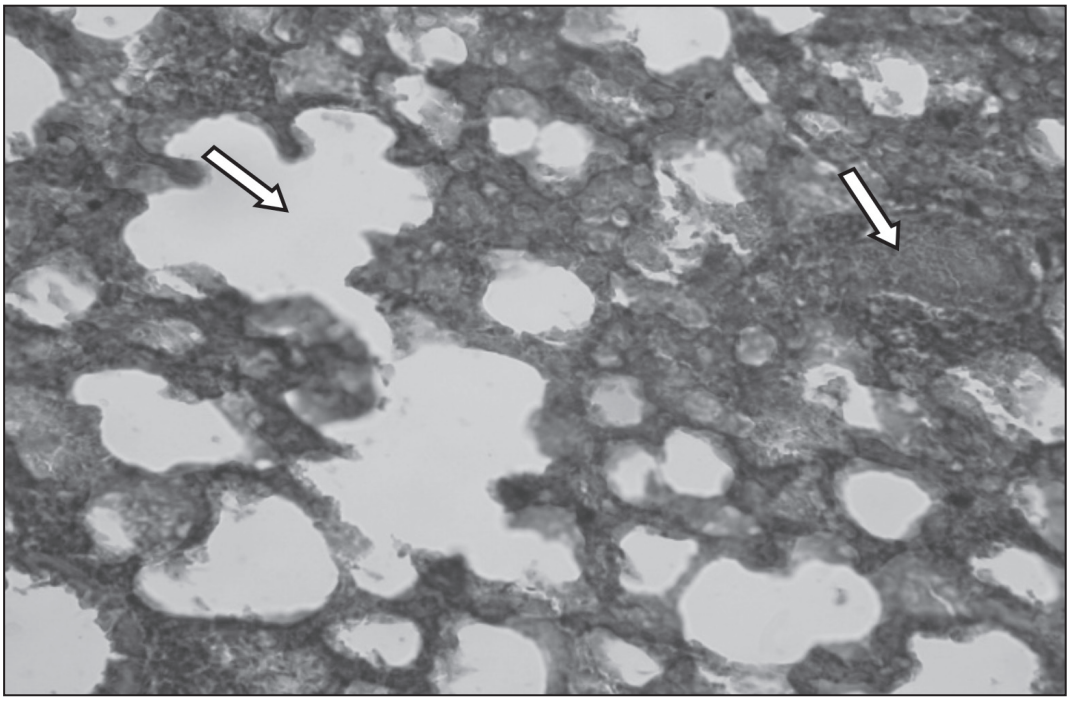

Figure 3. Emphysema, atelectasis and congestion in lung. Group III (day 11). (H&E, 20x)

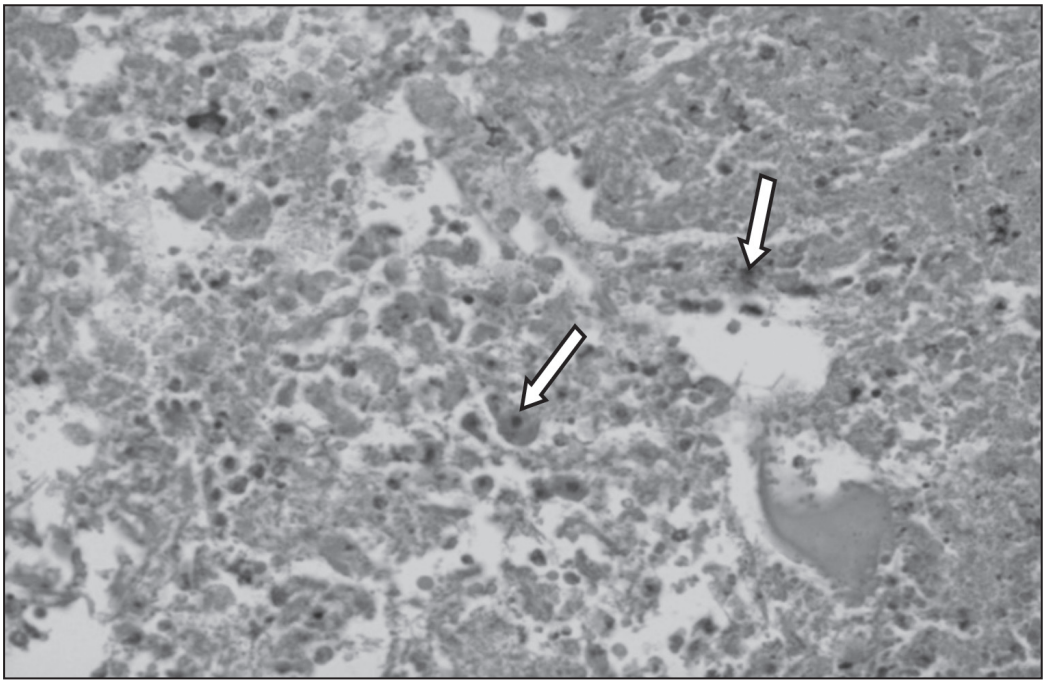

Figure 4. Necrosis of trophoblast in G III (H&E, 40x)

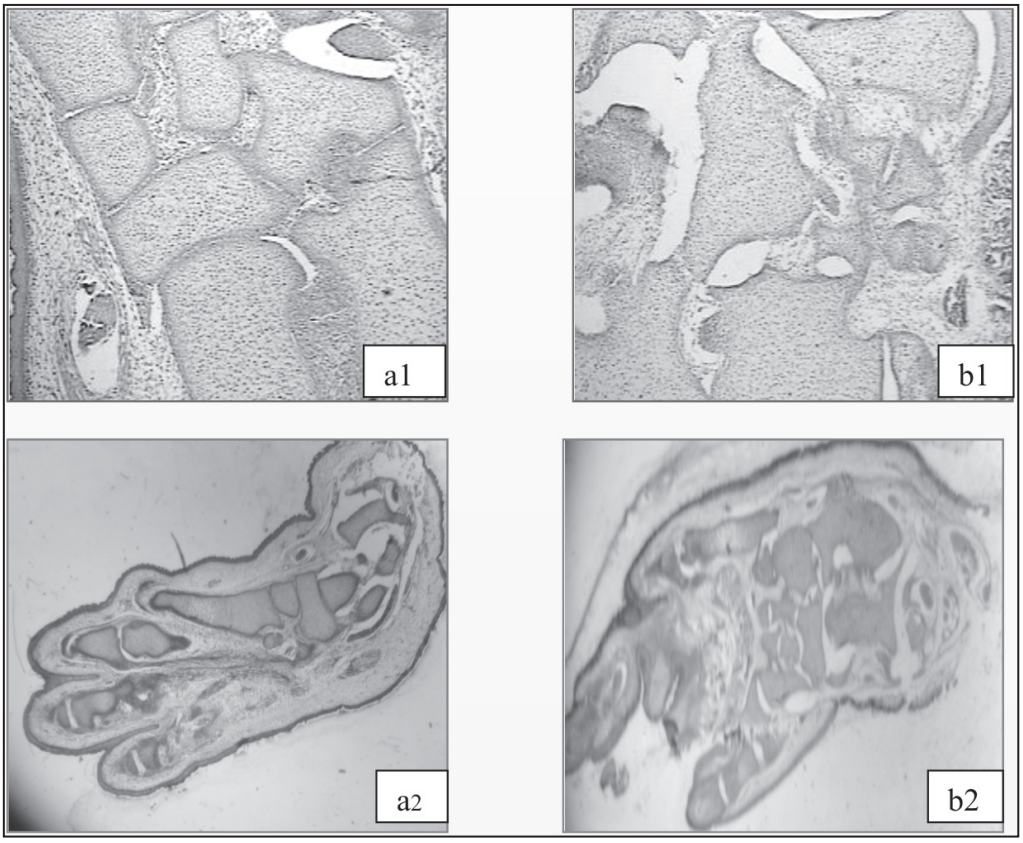

Figure 5. Hinlimbs from (a) control and (b) contaminated fetuses. (a1 and b1, 20x stained with H&E; a2 and b2, 10x stained with Masson technique).

al. to produce *in vivo* teratogenic effects in rats[49].

Cadmium can produce placental alterations[29, 36, 44]. In our experimental model, Cd was administered to rats on days 7, 9 or 11 of pregnancy since in this species, on day 7, implantation is complete and the vitelin placenta begins to form; on day 9, the formation of the corio-vitelin placenta is complete, and, on day 11, the corio-alantoid placenta is developing[4, 9, 10].

A high percentage (50 al 75%) of Cd deposited in the organism is found in the liver and kidneys[8, 20, 48].

The concentrations determined in these organs in the present work are in agreement with those reported elsewhere [6,40]. The deposition of the element produces hepatotoxicity and nephrotoxicity[3, 6, 15, 42, 48].

The pathogenesis of these alterations could be hypothesized as follow: the liver is the first organ to take most of the absorbed Cd[1, 48], inducing the synthesis of intrahepatic metallothionein (MT-Cd) in order to avoid the Cd cytotoxic effect in that cells[19, 29, 30, 32].

Hence, Cd reaches the kidneys where the MT-Cd complex is filtered. The epithelial cells lining the convoluted proximal tubules take by endocytosis a fraction of the filtered MT-Cd complex[47].

This endocytosed fraction would be responsible for the cellular lesions in the kidneys.

It has been shown that the kidney is the most sensitive organ to Cd intoxication[1, 20]. It is characterized by glomerular lesion and cellular degeneration in the proximal convoluted tubules, these determining alterations in the renal function[20].

The Cd values found in liver and kidneys of treated Cd groups would support the above described process. For example, in Group I, Cd was probably early deposited and later

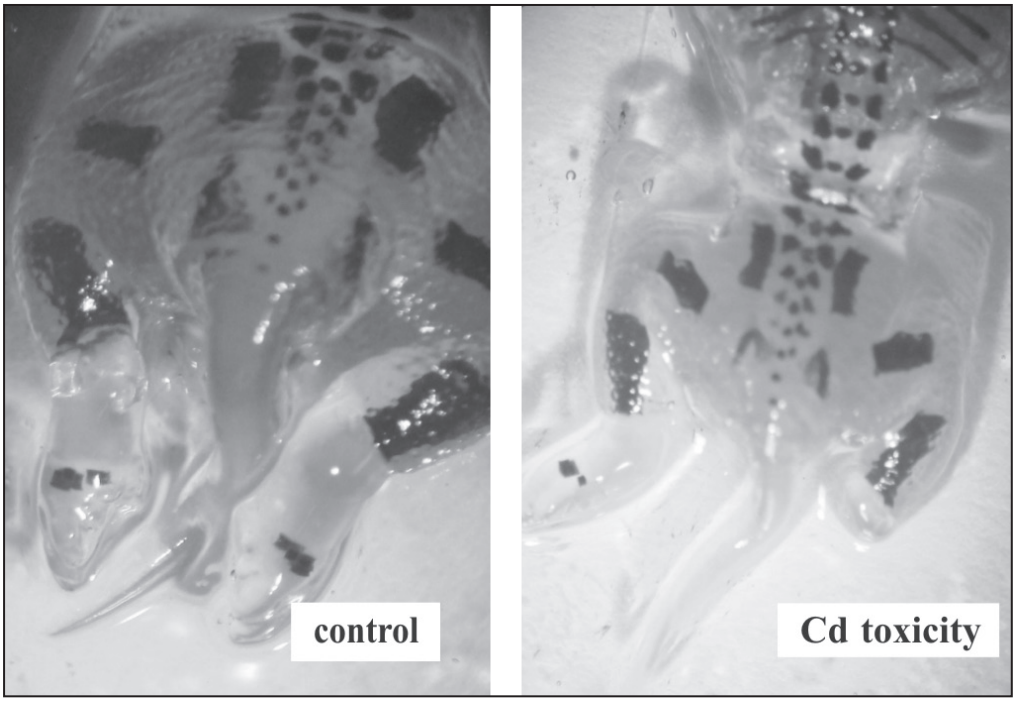

Figure 6. Different grades of ossification in limbs from fetuses of Control and Cd contaminated groups. (Allizarin technique)

eliminated in a great percentage, since the time between treatment and sacrifice was greater that in the other groups.

Concentrations of Cd determined in the spleen and lung[37] of animals treated with this heavy metal show increments that could be explained by the function accomplished by the studied organ. The results obtained in the present study are in agreement with those reported by other autor[37].

The spleen, among other functions, acts as a blood reservoir and as the place for hemocatheresis[17] while the lungs acts as an important filter of the blood[16, 37], and can retain little clots. It can also be considered as a blood reservoir, since it posses a great vascular net that can contain a great volume of blood at a given time.

In the spleen, a direct relationship between Cd concentration and time from treatment to sacrifice was established. It could be due to the incorporation of the heavy metal into the erythrocytes of treated animals.

In the lung, an inverse relationship between Cd concentration and time from treatment to sacrifice was determined. This is probably due to a higher renal clearance of the blood Cd in animals treated on day 7 of gestation (GI) than in the animals of the other experimental groups. Hence, the circulating Cd concentrations reaching the lungs would be low.

Concentrations of Cd in the placenta are directly related with the placental development stage[27]. The administration of Cd during the early gestation (GI and GII) determined that Cd passed the placental barrier and a greater percentage was later eliminated.

The placenta of animals in the GIII was more developed at the time of treatment, and Cd passed in a lesser extent and its tissue accumulation took place, which was responsible for the higher Cd concentrations observed[2].

The greatest percentage of Cd accumulated in the kidneys is located presumably in the proximal tubules[46]. The histological findings produced by the toxic effect of Cd in the kidneys and reported in the present work are in agreement with those reported elsewhere[24, 39].

The morphological alterations described here correspond to an acute/sub acute intoxication, since the experimental animals were sacrificed at 13, 11 and 9 days post-treatment (GI, GII and

GIII, respectively).

The death of hepatocytes of Cd-intoxicated rats can be due to necrosis[33].

The histopathological results obtained in the present study are similar to those reported in the reviewed bibliography[12, 28, 43, 45]. The severity of the morphological alterations would be directly correlated with the time elapsed between treatment and sacrifice of the experimental animals.

The administration of Cd to gestating rats produces necrosis of the placenta. Parizek[35] and Padmanabhan[34] observed that the administration of Cd on day 7 of gestation could determine severe placental malformations, since Cd could affect the ecthoplacental cone and the allantoids. The residual Cd in the maternal circulation could damage the placenta and pass through it causing teratogenesis[29].

The Cd deposits can affect the placental function[35]. According to the results of the present study, the severity of the lesions is in inverse relation with the stage of placental development, as it is described for animals of the GI.

The mean weight of placenta from intoxicated females was lower than in the control group, which is in agreement with other assays[12, 29]

A direct correlation could be determined between the low weight and the size of fetuses and the low weight of placentas of rats treated with Cd (GI, II and III).

Different authors have reported the effects of Cd on the fetal ossification process during pregnancy in rats[40] particularly in the vertebrae, ribs and metacarpal bones[8].

Skeletal alterations observed in fetuses from G II and III, could be due to the characteristic embryogenesis of the rat, where the intra-embryonic mesoderm extend on each side of the notochordal plate and specifically the beginning of somite formation takes place at the end of day 9 or during day 10 of pregnancy[21].

The formation of the intra-embryonic mesoderm and its differentiation in regions is fundamental for the formation of cartilaginous structures, which will be the base for the skeletal system development[21].

The administered dose of Cd determined increments in the tissue concentrations of the microelement and morphologic alterations in the studied organs, which are compatible with an acute/sub acute Cd intoxication[27, 45].

The dosage regimen described in the present article can be used for studying morphological and functional alterations produced by Cd intoxication in rats.

Further research is required to confirm some of these results and to understand some of the mechanisms involved on the development of placental and fetal alterations induced by the cadmium administered during the gestation.

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