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Teratogenic effect of cadmium on the foetal development of albino rats

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ABSTRACT
Foetus of pregnant rats exposed to single intraperitoneal injection of 2 mg/kg body weight cadmium sulphate on eighth gestational days; were compared with control (saline injected) fetuses to assess the effects of cadmium sulphate on foetal growth and ossification. Cadmium sulphate caused significant reduction in body weight and morphological abnormalities such as exencephaly, gastroschiasis, kinky tail and club foot. Cadmium reduced the length of long bones and caused a retardation of ossification of the centra of thoracic and lumbar vertebrae as well as delay in the formation of caudal vertebrae and intervertebral discs.

KEYWORDS: cadmium, foetus, skeletal abnormalities.

INTRODUCTION

The gradual increase in the industrial wastes, agricultural human impacts and chemical pollutants, are leading to a continuous increase of harmful effects on the environments, cadmium vapor and fumes account for most inhalation exposures, but dusts of repairable size (less than 10 μm) can also inhaled (Friberg 1985), lethal exposure has been estimated at so mg Cd/m^3 for one hour with regard to cadmium oxide dust and about half of that for fume (Friberg *et al.* 1971), cadmium may be absorbed through inhalation or ingestion, after inhalation 10-40% may be absorbed in man, the average oral absorption of cadmium amounts about 5% (Rahold *et al.* 1972) depends on the solubility of cadmium salt (Brown *et al.* 1978) also Gastrointestinal absorption is usually less than 10% increased in the presence of iron, protein, calcium or zinc deficiencies (Amdur *et al.* 1994).

Pollution by heavy metals is also destructive and has a very toxic effect on human heath, it has been established that certain metals are toxic to embryonic and foetal tissues which induce teratogenecity in multiple species (including humans) (Domingo 1994) also the effect of cadmium as a placental toxin in multiple species reported (Padmanadhan 1986).

On the other hand, cadmium can induce morphological malformations in multiple species; in hamster embryos malformations included exencephaly, microphthalmia, cleft lip, cleft palate, gastroschiasis, and umblical hernia which depend upon the stage of organogenesis during which cadmium exposure occurs (Samarawickarama & Webb 1981), but consist primarily of craniofacial and limb defects when the metal is injected intravenously (Gale & Horner 1987), in addition, it has been found that cadmium sulphate induced congenital microphthalmia in rats when administered to the mother on gestational days 8,9 or 10 (Takeuchi *et al.* 1979). Regarding the toxic effects of cd_2 on the skletal system, in vitro Dohi *et al.* (1993) studied the effects of cadmium on osteogenesis, they found that the histological examinationshowed a decreased area of cartilage and bone foci, also Wang *et al.* (1994) suggested that cadmium in conjunction with ca deficiency in adult mouse can induce an extreme demineralization characteristic of Itai–Itai like syndrome.; moreover it has been found that interaction of cadmium with calcium in skeletal system of adult rats produced osteodystrophies, low bone mass and increased incidence of frature (Goyer 1997).

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In adult ovarictomized rates, the effect of cadmium on the haversian system showed a large degeneration and necrosies (Li *et al.* 1997); in chickens exposed to cadmium showed a calcification disturbance of cartilage, osteoprosis, osteolysis and bone marrow aplasia (Bokori *et al.* 1995), In *Xenopus laveis* embryo cadmium chloride caused malformation of metal exposed frogs included sacropelvic and hind limb deformities (Plowman *et al.* 1994), in grass carp (*Ctenopharyngodon idella*) cadmium induced skeletal disorders, retardation of skeletal elements and decreased calcium content of the whole larvae (Lashein 2001).
Although several investigators have studied the toxic effects of cadmium on different species and human, yet there is a paucity of information regarding its specific effect on the skeletal system of rat foetus.

The aim of this study is to give more insight to the toxic effect of cadmium sulphate on the foetal growth, long bones and its ossification, and ossification disturbance of vertebral column of rat foetus.

MATERIALS AND METHODS

Females albino rats obtained from National Research Center, Cairo, Egypt, females rats weighing 150-200 gm. The females are introduced into the male's cages. The animals are paired, usually in the early afternoon. The following morning of every subsequent morning of the mating period vaginal smears are examined. The presence of sperms in the vaginal smear indicated successful mating and was considered (Zero) hour on day 0 of gestation, then the females are separated from the males, their body weight are recorded.
The pregnant animals were divided into 2 groups (10 animals in each group), the 1st group served as a control and were injected with 0.9 % NaCl at the 8th day of gestation, the 2nd group were injected with a single intra peritoneal dose 2 mg/ kg of $CdSo_4$ in the 8th day of gestation. All the animal were killed on 20th day of gestation. The selection of this dose has been carried out according to pilot tests. The foetus removed from the uterus weighed and examined microscopically for external malformations subsequently fixed in 10% formalin for studying the skeleton by double staining of cartilage and bone with alcian blue and alizarin (Mcleod 1980). The long bones of stained embryos and its ossified parts in the control and treated groups measured with a calibrated ocular micrometer fitted in microscope.
The results are represented as mean ± S.E and statistically analyzed using the unpaired student t-test.

RESULTS

Effect of cadmium on foetal development: Effect of cadmium administered in early gestation periods on foetal body weight and length of long bones are represented in figures 1-11. The fetuses of pregnant rats which exposed to single interaperitoneal injection with 2mg/kg $CdSo_4$ on the 8th day of pregnancy significantly decreased the foetal body weight with percentage 27% comparing with the control group. (Fig. 1). Examination of the morphological structure revealed that miscellaneous malformations among fetuses of treated groups. These malformations include exencephaly, club foot, gastroschisis and, short and kinky tail (Fig. 2).
Effect of cadmium on foetal skeletal system: The fetuses treated with cadmium at the 8th day of pregnancy exhibited reduction of the length of long bones of humerus and ulna and significant decrease in ossified parts for humerus and ulna with percentage of ossification –26% for humerus and –33% for ulna (Figs. 3 & 5).
Injection with $CdSo_4$ caused decrease in total and ossified parts of long bones of the hind limb, femur and tibia with –20% of change in the ossification, for treated femur and -29% for

treated tibia (Figs. 4 & 6). Moreover, retardation in the ossification of thoracic and lumber vertebrae, (Fig. 7), delay in the formation of intervertebral discs,and incomplete ossification of centrum vertebrae and deformed sacral and caudal vertebrae (Figs. 8, 9 &10).

Figure 1: Effect of $CdSO_4$ on foetal body weight.**$P \leq 0.01$

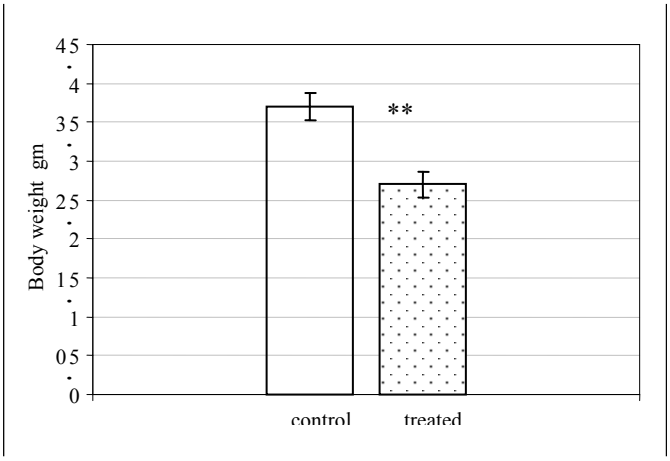


Figure 2: lateral view of 20 days old rat foetus head (1), abddomen (2), foot (3) and tail (4).
a: Control, b: Malformed showing head excencephaly (1), gastroschisis (2) and club foot (3).
c-d: Dorsal view of malformed rat foetus, c: excencephaly (arrow). d: club foot (arrow).
e-f: Verntral view of malformed rat foetus. e: 1- Gastroschisis 2- club foot. f: kinky tail (arrow).

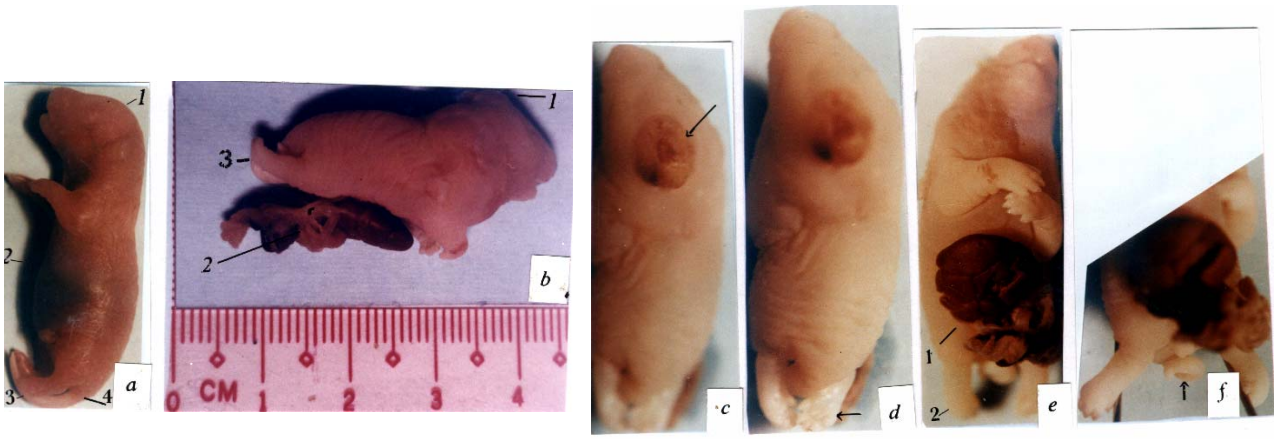


Figure 3: Effect of $CdSO_4$ on the length of humerus and ulna of rat foetus. (*$P \leq 0.05$ & **$P \leq 0.01$).

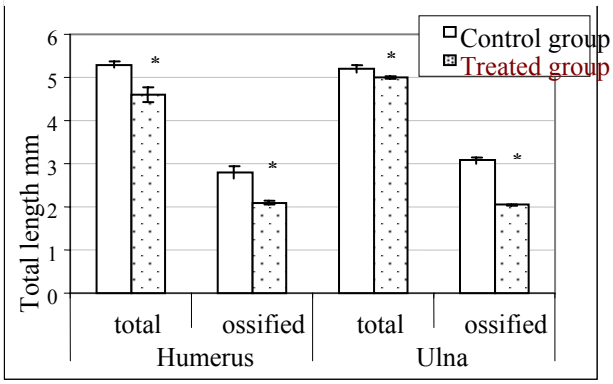
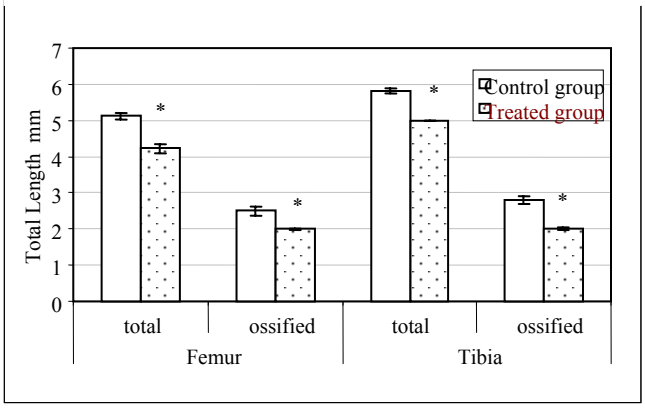


Figure 4: Effect of $CdSO_4$ on the total length of tibia and femur of foetus. (*$P \leq 0.05$ & **$P \leq 0.01$).



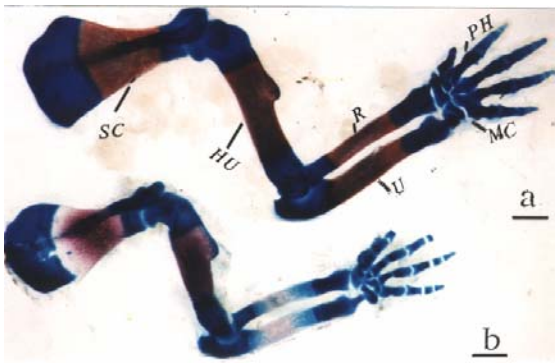


Figure 5 (X=14):
a: The scapula (sc) and the fore limb of control foetus showing scapula (sc) humerus (HU), radius (R), ulna (U) metacarpals (MC) and phalanges (PH).
b: showing decrease in ossifying length of humerus, ulna and phalanges (PH).

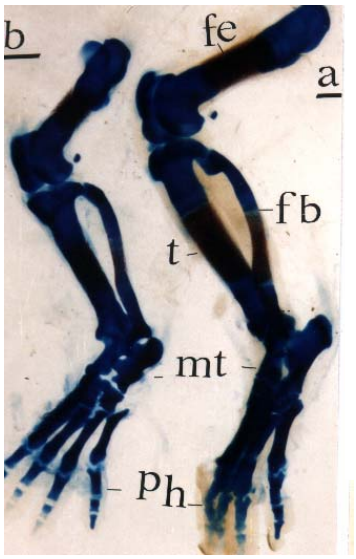


Figure 6 (X=14)**:**
a: The skeleton of the hind limb of control foetus showing femur (FE); tibia (T); fibula (FB) metatarsus (MT) and phalanges (PH).

b: The skeleton of the hind limb of maternally treated foetus showing shortness of the bones of femur, tibia and fibula.

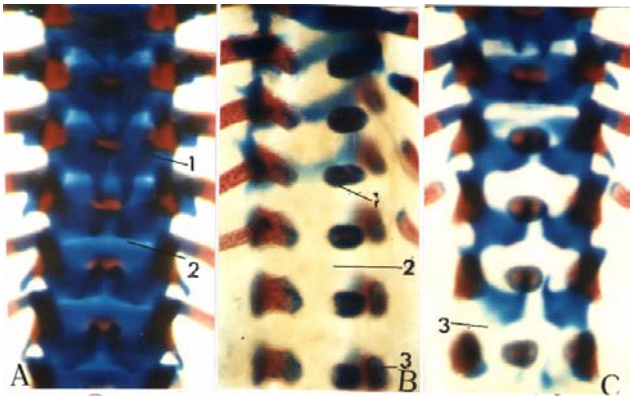


Figure 7 (X=14): dorsal view of control fetal thoracic and lumbar vertebrae showing:
a: normal vertebrae with well ossified centra. (1) centrum of a thoracic verebra; (2) inter-vertebral disc; (3) first lumbar vertebra.
b-c: showing incomplete ossification of the vertebrae with absent of centrum of thoracic vertebrae and of intervertebral disc; incomplete formation of vertebral body and first lumber vertebra

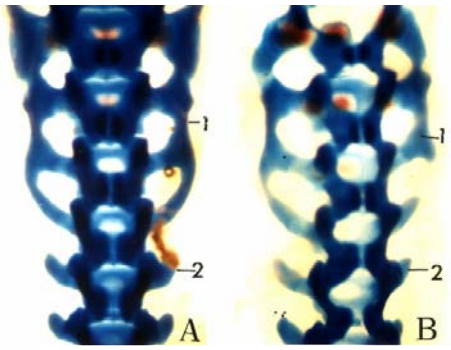


Figure 8 (X= 14): (A) ventral view of control fetal sacral and caudal vertebrae showing (1) sacral vertebrae (2) caudal vertebrae.
(B) Maternally treated fetal vertebrae showing deformed sacral and caudal vertebrae (1) as well as delay in ossification of centrum (2).

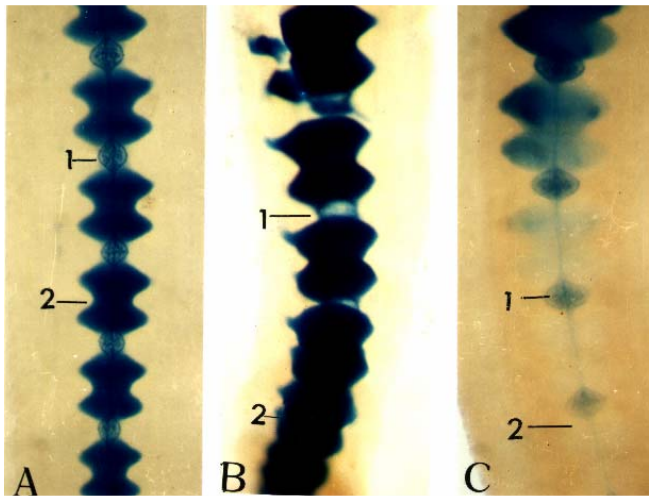


Figure 9: (A) caudal vertebrae of rat foetus. A: control showing normal caudal vertebrae; B-C: Maternally treated showing curved caudal vertebrae and incomplete ossification.

Figure 10: A: normal vertebral column of rat foetus; B-C: Maternally treated showing curved vertebral column & incomplete ossification.

DISCUSSION

It well known that acute and sub acute exposure of experimental animals to cadmium, induces a pro-oxidation state in the peroxidation of membrane polyunsaturated fatty acids, peroxidation process, resulting in the production of lipid radicals and in the formation of a complex mixture of lipid degradation products extremely toxic of the cells (Manca *et al.* 1990), Free radicals are evolved at stages of cadmium intoxication (Richelmi *et al.* 1989) which may be induced by indirect interactions between Cd^{+2} and critical cellular sites (Vincent *et al.* 1989), On the other hand, the effect of cadmium in depleting cellular Glutathione reductase would disturb the redox status of the cells. Perturbation of the redox potential of the cell may alter many important functions including biosynthetic reactions (Hazelton & Lang 1980). This may explain the foetal growth retardation as well as skeletal anomalies which observed in the present study. The disturbance of Ca hameostasis is a possible mechanism for oxidative cell injury (Orrennius *et al.* 1989). Calcium has been reported to stimulate numerous enzymatic pathways and therefore rapid fluctuation in the concentration of this cation in the cytoplasm which will disturb the metabolic status and may thus contribute to the cytotoxicity of heavy metals especially cadmium (Wang *et al.* 1994).

It is well established that maternal dietary consumption of cadmium throughout gestation induced foetal growth retardation in laboratory animals (Webster 1978). In the present investigation, cadmium sulphate administrated to pregnant female rats early gestation caused significant reduction in body weight. Foetal growth or protein synthesis may be more sensitive to the toxic effect of cadmium than cell proliferation, protein concentration and protein DNA ratios were reduced at a lower drinking water cadmium concentration than total DNA (Ahokas *et al.* 1979) concluded that accumulation of only small amounts of parenteral cadmium would inhibit embryonic DNA and protein synthesis.

The role of the placenta in normal embryogenesis is an important fact, but the correlation between placental abnormalities and foetal dysmorphogenesis have been recognized (Padmanbhan 1986). Also cadmium is associated with alteration in placental function, ranging from decreased transport of nutrients to necrosis (Wier & Miller 1986).

Ahokas *et al.* (1979) observed that cadmium crossed the placental barrier in laboratory animals in early gestation. Transport of even smaller quantities of the metal into the embryo during early gestation could cause severe malformations. On the other hand, Cadmium administered at the 8^{th} day of pregnancy caused marked reduction in the length of long bones of rats. It is known that embryonic bones are derived by oxidative pathways from mesenchymal and ectodermal differentiation, mesoderm differentiation is dependent on succinic oxidase and folic acid while ectoderm differentiation is dependent on glucose and sytochrome oxidase (Runner & Dagg 1960). It seems possible that skeletal anomalies reported in the present investigation may be due to the inhibitory action of Cadmium on cytochrome oxidase and are similar to the results obtained by Dohi *et al.* (1993) who suggested that Cd_2 administration inhibits the osteoblastic and chondroblastic differentiation pathway in bone marrow through direct effects on these cells. This coincide as well with Lashein (2001), who reported that heavy metals, Cadmium and Lead, exerted severe degeneration of the chloride cells in the skin and gills of both early and older larval stages of grass carp (*Tenopharyngodon idella*) resulting in poor deposits of skeleton.

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الملخص العربـــى

تشوهات أجنة الجرذان الناتجة عن تأثير الكادميوم

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توضح الدراسة التأثير السام لكبريتات الكاديوم على نمو أجنة الجرذان وتكوين الهيكل العظمى حيث تم حقـن الأمهـات الحوامل بجرعة واحدة من كبريتات الكادميوم (2 ملى مج/ كجم) في اليوم الثامن من الحمل بداخل التجويـف البريتـونى ومقارنتها بأمهات حوامل تم حقنها بجرعة واحدة من محلول ملحى بداخل التجويف البريتونى في اليوم الثامن من الحمـل0 وقد سبب الكادميوم نقص في وزن الأجنة وكذلك سبب تشوهات في الشكل الخارجى مثل بروز المخ من الجمجمة وخلـع جدار البطن وقصر والتواء الذيل وحنف القدم المدببة وبفحص العينات المصبوغة لإيضاح تكوين الهيكل الداخلى ثبت أن حقن الأمهات الحوامل في اليوم الثامن بكبريتات الكادميوم (2ملى جم/ كجم) يؤدى إلى نقص في طول العظام وتأخر تكلس مراكز التعظم للفقرات الصدرية والقطنية والعجزية والذيلية وكذا تأخر تكوين الأقراص الغضروفية بين الفقرات.