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Original Article

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Effects of Nano Zinc Oxide on the Hind Limb Bud of NMRI mouse embryos

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Abstract

Nanomaterials are particles with 10 to100 nanometer size. They can easily be transported through the skin, lung alveoli, and placenta. Materials in nano scales show different properties compared with the same materials in macro or larger scale. It is found that zinc element is an essential metal for normal physiological functions. However increase or decrease of this element during pregnancy, for example with using cosmetics and deodorants, can be teratogen and cause several fetal abnormalities. Given this fact, this study was conducted to investigate the effects of nano zinc oxide on the developmental stages of hind limb budsin NMRI mice embryos.

Keywords: Nanomaterials, nano zinc oxide, hind limb bud.

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Introduction

Zinc requirement of the body increases during the latter half of pregnancy and alongside with the rapid growth of the fetus (Walshetet al., 1994). In birds, fish and mammals, zinc deficiency can lead to irregular embryonic development (Apgar, 1985). The rat is a well-studied species with regard to the effects of zinc deficiency on pregnancy (Vallee et al.,1970). While human embryo limb bud is formed during the fifth week of pregnancy, in fetal mice, limb bud is formed during ninth or tenth week of pregnancy. Hands and feet are formed is an area called the Bud Zone(Gilbert, 2000). Advances in nanotechnology have attracted several investigators to take the advantages of the unique properties of nanomaterials and nanostructures in development of novel therapeutic approaches. Zinc Oxide (ZnO) is an inorganic compound with a wide industrial application (Hernandez-battez et al., 2008). Due to their small size, nanomaterials can easily be transported into skin, lung alveoli and placenta (JuNamet al., 2008). Regarding the direct relationship between mother and fetus, the transported nanomaterials can easily cross the biological barriers and exert their effects on the development of fetal organs. This study aimed to investigate the impact of nano-ZnO on mice fetal development.

Methods

The experiment was conducted on pregnant female mice. The day after mating, female mice was considered as day zero of pregnancy on which observation of vaginal plug was started. At day 11 of gestation, the female mice were intraperitoneally injected solutions of two different doses of nano ZnO (particle size of 20 nm, and purity of 99%).Two experimental groups were considered. Sterile water was selected as the solvent. While the control group received no injection, the sham group was administered 1.5ml of the solvent. The nanoZnO was injected based on grams per kilogram (g/kg/bw). Materials were weighed in a glass test tube with 1.5ml of distilled water. Figure1 shows the TEM and SEM images of nanoZnO. The white liquid with an insulin syringe for injection to the animals were transferred to the animal room, and a solution of nano zinc oxide was then injected into the peritoneal cavity of mice. On the day 15 the pregnant mice were anesthetized by ether and the fetuses were removed from the uteri. The embryos were transferred to petri HBSS. Hind limbs of the fetus were separated under a microscope. The limbs were then put in Bouin fixator for two hours and then the rest was done by flooding and the tissue preparation (Parivaret al.,2008).The morphology and histology of each limb bud group were studied.

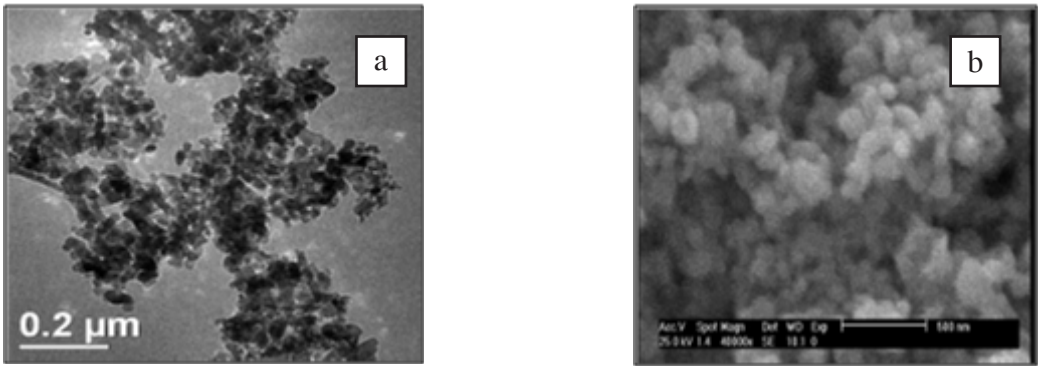

Figure 1: TEM (a) and SEM (b) image of nano Zinc Oxid and Properties of nano zinc oxide.

Finger, leg and thigh were studied in control, sham and experimental groups. Changes in the number of mesenchymal cells, cartilage cells (chondrocytes), atrophied, red blood cells, and muscle and bone cells were recorded. Five stages of oestrogens development, including Resting, Hypertrophied, Proliferating, Degenerating, and Osteoblasts, were studied. Data were analysed using one-way ANOVA followed by Tukey post hoc test. All statistical analyses were carried out using SPSS Version 18 Software.

In the test groups, the embryonic abortion was observed that can be explained by the presence of nano-ZnO (Gunson et al., 1982).Intra peritoneal injection of nano-ZnO in two test groups showed significant effects on hind limb bud development. In addition, several changes in the morphology of the limbs was observed after injection (dose of 1.5g/kg), especially in the second test group (Figure2, Tables 1-3).

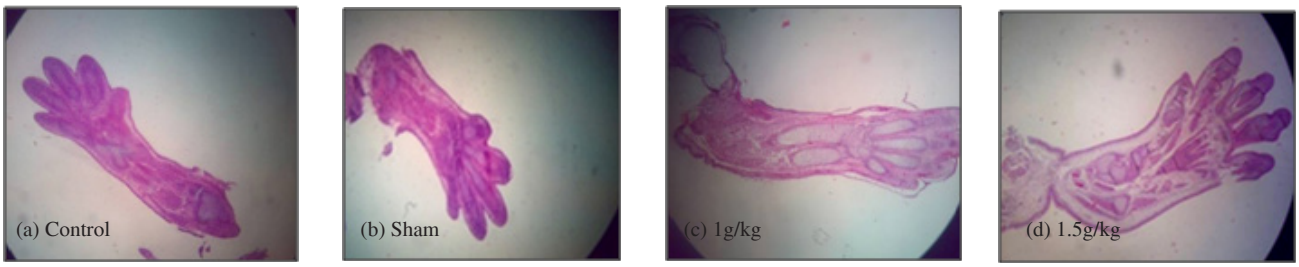

Figure 2: Photomicrograph of hind limb bud in control (a), sham (b), experiment 1 (c), experiment 2(d) X 100).

Table 1: Statistical analysis of cells in toe region in control, sham and experimental groups (X□ ±SEM)

Men±SEM	Mesenchyme	Chondrocyte	Atrophy	Red Cell	Muscle	Hypertrophied	Resting
control	100.00±6.54	4.50±0.94	5.00±0	6.25±0.81	2.70±0.49	0	0
sham	88.75±6.66	5.62±1.17	5.75±0.49	5.62±0.62	3.87±0.44	0	0
experimental 1	32.50±6.19 ***	*** 127.50±15.20	5.75±1.04	7.50±1.33	3.87±0.54	* 3.87±0.54	* 1.50±0.32
experimental 2	43.12±1.61 ***	*** 77.50±11.29	*** 11.25±1.25	11.00±2.55	*** 8.12±1.31	*** 8.87±1.74	*** 3.87±0.54

(*P <0.05 ,** P< 0.01,*** P< 0.001)

Table 2: Statistical analysis of the number of cells in Leg region in control, sham and experimental groups (X ±SEM). Asterisks above numbers indicate significant increase and at the bottom indicate significant reduction in the cell numbers.

Men±SEM	Mesenchyme	Chondrocyte	Atrophy	Red Cell	Muscle	Hypertrophied	Resting	Proliferating
control	90.00±5.00	99.37±1.13	6.25±1.25	5.87±0.97	18.75±2.26	0	0	0
sham	72.50±8.18	12.50±0.94	6.25±0.81	6.87±1.31	19.62±2.41	0	0	0
experimental 1	66.25±7.71	** 23.75±4.12	3.87±0.39	4.62±0.37	31.25±4.79	7.51±1.33	0	0
experimental 2	22.50±3.65 ***	6.87±0.91	8.75±1.56	11.87±2.30	* 44.37±11.07	*** 7.00±5.00	*** 22.50±2.67	*** 8.75±1.25

(*P <0.05 ,** P< 0.01,*** P< 0.001)

Table 3: Statistical analysis of the number of cells in Thigh region in control, sham and experimental groups (X ±SEM).

Men±SEM	Mesenchyme	Chondrocyte	Atrophy	Red Cell	Muscle	Hypertrophied	Resting	Proliferating
control	103.75±2.63	12.50±0.94	7.37±1.37	3.50±0.56	21.25±1.25	20.00±2.67	0	0
Sham	90.62±2.39	14.37±1.47	8.12±1.31	3.87±0.54	20.62±1.99	23.75±1.82	0	0
experimental 1	23.50±5.90 ***	** 23.75±4.19	4.87±0.87	4.87±0.47	38.75±3.98	*** 55.00±5.34	*** 4.87±0.89	*** 6.37±1.7
experimental 2	21.87±2.30 ***	3.12±0.54 *	9.37±1.75	** 9.37±1.99	*** 69.37±8.26	10.62±1.40	** 2.00±0	2.00±0

(*P <0.05 ,** P< 0.01,*** P< 0.001)

Discussion

Increased level of zinc metal especially during the pregnancy periods may lead to an increase in hormonal function and mitogenesis, especially in embryonic development. This process is conducted by signal transduction, gene transcription, and finally increased rate of RNA synthesis (Mac Donald, 2000). Zinc also plays an important role in the zinc finger proteins. Zinc fingers coordinate zinc ions by building a bridge between cysteine and histidine. These proteins individually bind to DNA and accelerate the transcription process (Miller, 1985). Nanoscale materials can enter the fetus body because of their ability to easily cross biological barriers. The results of this study showed that the nano ZnO can affect the developmental stage of limbs by accelerating the developmental phase of these organs. Changes in chondrocyte and mesenchymal cells in the hypertrophic cartilage and at rest and proliferating cells were considered as the steps of pre-ossification. We showed in experimental test groups 1 and 2 the important role of these cells in controlling cell division, proliferation and differentiation. For example, a significant decrease ($P < 0.001$) in the number of mesenchymal cells in experimental groups1 and 2 show the conversion of these cells into cartilage cells and bone. Wanga has demonstrated that the differentiation of mesenchymal cells into bone depends on presence of zinc ions (Wanga et al., 2007). Also Ganson showed that mammals poisoned with high zinc concentrations are generally subjected to a decreased growth rate, subcutaneous hematoma, ulcers, hemorrhoids and articular lesions (Ganson et al., 1982). In severe anemia, accumulation of ZnO on the muscles and tissues, especially in the pancreas and kidney was observed. In addition, according to our results, it seems that accumulation of nano ZnO in different organs of the mother or fetus can lead to the abortion. The unusual growth of limbs, in terms of cartilage and bone formation in test group compared to the sham and control groups shows that ZnO nanoparticles could negatively impact the normal development of hind limbs during pregnancy.

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