Journal of Pioneering Medical Sciences
Received January 05, 2024 Accept March 05, 2024 Publish April 29, 2024.
Volume 13, Issue 2, Pages 193-197

DOI https://doi.org/10.61091/jpms202413229

Pathological Alterations Induced by Nickel Oxide Nanoparticles in Pregnant Mice and Their Embryos

Hafidh I. Al-Sadi[1,*], Hani Malullah H. Al-Hamdany[2] and Seema' Sami Al Mudaris[2]
[1]College of Pharmacy, The University of Mashreq, Baghdad, Iraq.
[2]Department of Basic Sciences, College of Basic Education, University of Mosul, Mosul, Iraq.

Corresponding author: Hafidh I. Al-Sadi (e-mail: hafidh19477@gmail.com).

Abstract The purpose of this study was to investigate the effects of nickel oxide nanoparticles on pregnant mice and fetal development. Nickel oxide nanoparticles (<50 nm) were injected intraperitoneally (1, 10, 20 mg/kg) in pregnant mice daily from the 7th -18th gestation day. At the 18th gestation day, the mice were euthanized, necropsied, and pathological examination of the mice and their embryos was done. Exposure to the nickel nanoparticles induced an increase (low and medium doses) and a decrease (high dose) in the activity of the mice. Abortion was recorded at the rates 10%, 20%, and 30% in groups treated with doses of 1, 10, and 20 mg/kg, respectively. Fetal malformations were noted at rates of 60%, 70%, and 75%, for the doses 1, 10, and 20 mg/kg, respectively. They included arrested growth, absence of the extremities, C-shaped fetuses, anencephalon, myelocele, exophthalmia, spina bifida, absence of facial details, absence or deformities of the tail, micromelia, cyanotic skin, abdominal hemorrhages, and meningocele. Microscopically, degenerative, necrotic, circulatory, and inflammatory lesions in the kidneys, liver, lungs, brain, and heart were noted in pregnant mice. In the embryos, there was incomplete growth of the renal, nervous, and pulmonary tissues. Additionally, there were degenerative, necrotic, and circulatory changes in the kidneys, liver, brain, lungs and heart. It was concluded that exposure to intraperitoneally administered nickel oxide nanoparticles (<50 nm) induces abortion and toxic lesions in the kidneys, liver, brain, lungs and heart of pregnant mice; and fetal malformations and pathological lesions in the embryos.

Key Words nanoparticles, pregnant, spina bifida, Abortion, embryos

1. Introduction

Nickel is a silver white metallic chemical element naturally present in the earth's crust. This element and its compounds are widely used in industry because of its unique physico-chemical properties such as being tough, harder than lron, ferromagnetic, having good plasticity and highly resistant to rusting and corrosion [1]. Animal studies have shown the presence of an association between nickel deprivation and decreased growth, reduced reproductive rates, and alterations of serum lipids and glucose [2]. Nickel is potentially harmful element to humans and human exposure to the element or its compounds has the potential to produce a variety of pathological effects, which may include cutaneous inflammation such as swelling, reddening, eczema, and itching, and may also include allergy reactions and teratogenicity [3]. Fibrosis of the lungs and lung cancer are the most important adverse health effects of nickel exposure. In humans, epidemiological studies have indicated that occupational exposure to nickel increased the incidence of some types of cancers, such as lung, head, neck and nasal cancers. The International Agency for Research on cancer has long been classified nickel compounds as human carcinogens [3]. Furthermore, excessive doses of nickel micro-particles (NiMPs) induces reproductive toxicity. Nickel ions have variety of adverse effects on reproduction and development, including influence on male and female subfertility or fertility, abortions, malformations, and birth defects. [2] found that NiMPs treatment can decrease the reproductive capacity of zebrafish. Soluble nickel salts have been shown to disturb mammalians and model organism reproductive functions. The reproductive toxicology of nickel may be mediated mainly through hormonal effect, both at neuroendocrine and gonadal levels in the hypothalamic-pituitary- gonadal (HPG) axis [4].

Nanomaterials are defined as materials with at least one dimension ranging from 1 to 100 nm, have unique or even increased physicochemical properties, such as nanoscale size

effects, quantum effects, expanded surface area as well as unique electric, thermal, mechanical and imaging properties. Among the most widely used nanomaterls are the metallic nanoparticles including metallic nickel nanoparticles (NiNPs). NiNPs could lead to the formation of a product with many new characteristics, including a high level of surface energy, high magnetism, low melting point, high surface area, and low burning point. NiNPs have been shown to induce liver and spleen injury, lung inflammation, cardiac toxicity and exhibit higher carcinogenic potential than fine particles [5]. In has been reported that NiMPs have reproductive toxicity [6]. In comparison with NiMPs reproductive toxicity, NiNPs toxicity was more severe [3]. Recently, [7] found that exposure time- dependent differences in the toxicity of nickel nano and microparticles in the ovary of rats. Nano nickel was cumulative in the ovaries and affected steroidgenesis. Furthermore, increased generation of reactive oxygen species and enhanced oxidative stress were found to contribute to cytotoxicity. The goal of this study was to evaluate the effects of intraperitoneally administered NiONPs on pregnant mice and their embryos.

2. Materials and Methods

A. Chemicals

Nickel oxide nanoparticles (green black powder, 99.8% purity, <50nm particle size, CAS number: 1313- 99-1, MDL number: AFCD0001445, molecular weight:74.69, Density: 6.67 g/mL, Bulk dersity: 0.51 g/mL) were purchased from Sigma- Aldrich, China. All other chemicals used were of the highest grade commercially available.

B. Animals

BALB/C mice, weighing 23 ± 2 gm were obtained from College of Medicine, University of Salahuldeen (Erbil Province, Iraq). The animals were housed in plastic lab animal cages in ventilated room, which was maintained at 24 ± 2°C and 60% ± 10% relative humidity under a 12- hr light /dark cycle. Water and commercial laboratory complete food were provided ad libitum. Mice ready for fertilization were placed in separate cages (3 females with 1 male) overnight, and in the next morning, fertility was examined by observing the vaginal plug. The day of mating was the zero day of pregnancy, and the day after was the first day of pregnancy [8].

C. Experimental Design

The experimental protocols confirmed to the ethical guidelines of the University of Mosul. Three groups of pregnant mice (n= 10 per group) were administered a daily dose of 1, 10 or 20 mg/kg nickel oxide nanoparticles by intraperitoneal injection, starting from the 7th gestation day to the 18th gestation day. In addition, 10 mice were administered an equivalent volume of distilled water as controls. Behavioral changes (signs) were carefully recorded daily after treatment.

D. Histopathological Examination

At the 18th gestation day, the mice were sacrificed, dissected, and the kidneys, liver, brain, lungs, and heart were excised and fixed in 10% formalin for 48 hours. Following fixation, tissue specimens were collected, washed, dehydrated, cleared, embedded in paraffin and sectioned at 4-5 μm thickness. The tissue sections were mounted on glass slides and stained with hematoxylin and eosin [9]. Examination and photography of the slides were done using a compound light microscope type Richert Neovar provided with digital camera type Olympus, OM- JAPAN. Final magnification of the photographs was calculated according to magnification of the ocular and objective lenses.

3. Results

Mice of the 1 and 10 mg/kg groups exhibited increased appetite, hyperactivity, and agrgressive behavior. In contrast. Mice of the 20 mg/kg group showed decreased appetite, decreased activity, and became isolated. Abortion occurred at the rate of 10%, 20%, and 30% in the 1, 10, and 20 mg/kg groups, respectively. Examination of the uterine horns revealed irregular distribution of the fetuses, congestion and atrophy of the uterine horns, and malformed fetuses. Malformations occurred at the rate of 60%, 70% and 75% in mice of the 1, 10, and 20 mg/ kg, respectively. They were in the form of elongated fetuses, arrested growth, absence of extremities, absence of eyes, encephalocoele, myelocoele, encephalomyelocoele, anencephaly, deformed trunk, deformities of the tail (aquiline tail), abdominal hemorrhage, enlargement of the skull, proptosis, curved vertebral column, and spina bifida (Figure. 1 and 2).

Histopathological examination of internal organs of the pregnant mice revealed cell swelling, necrosis and sloughing of renal tubular epithelium, hypercellularity of the glomeruli and hemorrhages in the renal interstitium (Fig. 3a). Sections of the liver showed degeneration and necrosis of hepatocytes, dilatation of sinusoids, presence of foci of mononuclear cell infiltration, and appearance of megalo- hepatocytes (Fig. 3 b and c). Sections of the brain demonstrated degeneration and necrosis of pyramidal cells, granular cells, and neuroglial cells (Fig. 3d). In the lungs, there were emphysema, hemorrhages, serous pneumonia, hyperplasia of alveolar cells, hypertrophy of smooth muscle cells in bronchiolar wall, and hyperplasia of the bronchiolar epithelium (Fig. 3 e and f). In the heart, there was vacuolar degeneration of some of the myocardial cells.

In the embryos, the hispathological lesions were incomplete growth of renal structures (glomeruli and tubules) and thickening of renal interstitium (Fig. 4a). The liver showed disarrangement of the hepatic cords and hepatic sinusoids, congestion, and appearance of hepato-meglaocytes (Fig. 4b). In the brain, there were hypercellularirty, congestion, and edema of the cerebral cortex (Fig. 4c). Sections of the lungs showed hyperplasia of the bronchiolar epithelium, thickening of the interalveolar septa, congestion of the interstitum, narrowing of the bronchioles, and the accumulation of blood

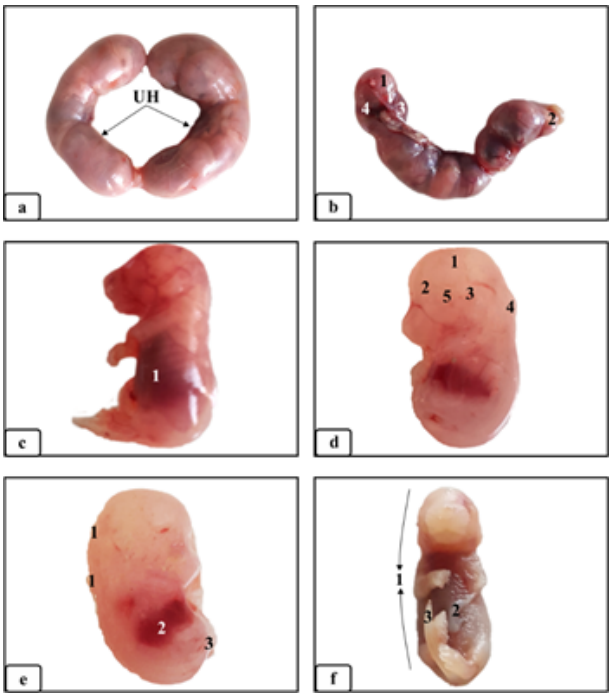

Figure 1: Uterine horns and fetuses of control and experimental mice. a) control uterine horns (UH), b) irregular distribution of fetuses (1), malformed fetuses (2), accumulation of fat- like material (3), hemorrhagic areas (4), c) control fetus, d) malformed fetus, (NiONPs), enlargement of head (1), absence of facial details (2), absence of auditory meatus, myelocoele (4), anophthalima (5), e) malformed fetus (NiONPs) showing short, arching, and swollen trunk (1), abdominal congestion (2), loss of the tail (3), f) malformed fetus (NiONPs), elongated and congested fetus (1), exterioration of the intestines (2), long curved tail with pointed end (3)

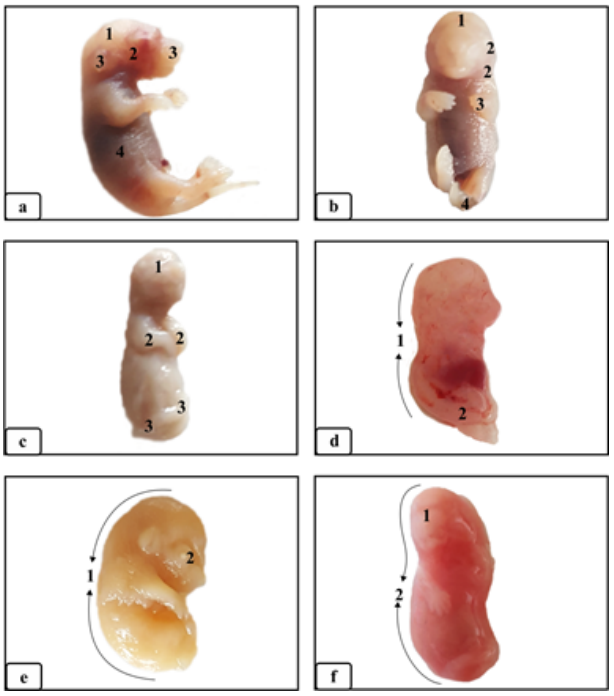

Figure 2: Maformed fetuses (NiONPs) a) swollen head (1), hemorrhages around the eyes (2) deformity of the facial details (3), congestion and cyanosis of the skin(4), b) rounded head (1), absence of the neck and auditory meatus (2) atrophy of the right forearms (3), few fingers in the right hind limb (4), c) anophthalmia (1), shortening of forearms (2), atrophy of the hind limbs (3), d) concave and congested trunk (1), atrophy of the posterior part (2), e) 'C'- shaped fetus (1), proptosis (2) f) tilted oval head (1), curved vertebral column (2)

in the thorax (hemothorox) (Fig. 4 d and e). In the heart, the myocardial cells were replaced by fibromuscular tissue (Fig. 4 f).

4. Discussion

The present study investigated the potential toxic effects of nickel oxide nanoparticles after intraperitoneal injection of 1,10, or 20 mg/kg daily (from 7th to 18th gestation days) in pregnant Balb/c mice. It was intended to provide information for establishing safety criteria with regard to human exposure and the toxic effects of NiOPs. Many studies have shown that cytotoxicity of metal- based nanoparticles is related to particle size, specific surface area, crystal conformation, exposure mode, and chemical components, etc [10]. Exposure to metal- based nanoparticles adversely affects the reproductive system of female animals.

In a study done by [11] continuous exposure of female mice to 2.5, 5 or 10 mg/kg-1 titanium dioxide nanoparticles via tube feeding was done. Within 30 days, the mice exhibited premature ovarian failure characterized by a reduction in reproductive ability with decreased levels of inhibin B, estradiol, and progesterone, among others. Exposure to metal-based nanoparticles induced unique biological effects at the cellular level by affecting transmembrane and basic cellular processes, such as cell division, proliferation, apoptosis, and regulation of signal transduction pathways [10]. Until now, no definite conclusions have been made on the cytotoxicity mechanism induced by metal- based nanoparticles. Oxidative stress and inflammation are the main explanatory factors for the cytotoxicity.

In this study, NiONPs injected intraperitoneally daily from the 7th gestation day to the 18th gestation day contributed greatly to the occurrence of abortion. This abortive effect of NiOBPs was dose- dependent since the percentage of abortion increased with increase of the dose (1,10,20 mg/kg). Nanoparticles are known to accumulate in the placenta and induce placental damage through generation of oxidative stress, inflammation, and alteration of gene expression [12]. From a review of 73 studies on placental translocation of particles (21 in vitro/exvivo studies, 50 animal studies, and 2 human studies), Bongaerts et al., [13] conclude that (i) (ultra) fine particles and engineered nanoparticles can bypass the

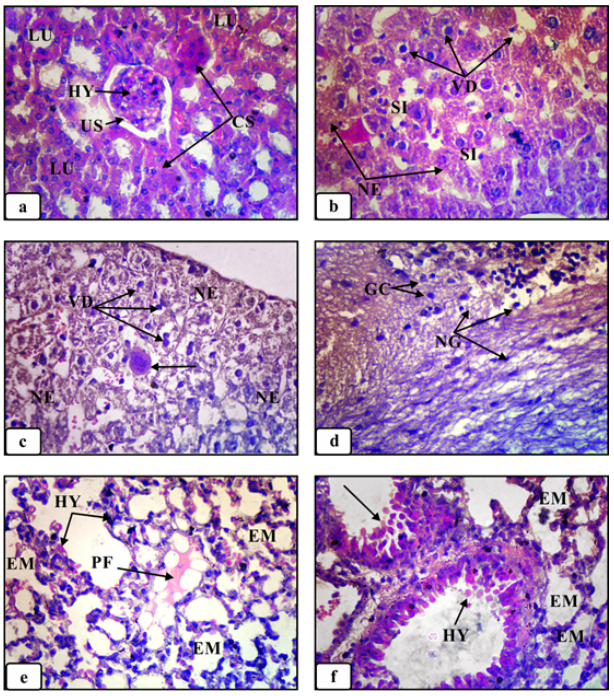

Figure 3: Histological changes in pregnant mice (NiONPs). kidneys, cell swelling of tubular epithelium (CS), hypercellularity of glomeruli (HY), narrowing of urinary space (US), b) liver, vacuolar degeneration (VD), necrosis (NE), dilatation of sinusoids (SI), c) liver, vacuolar degeneration (VD), necrosis (NE), appearance of hepatomegalocytes (arrow), d) brain, degeneration and necrosis of neuroglia (NG) and granular cells (GC), e) lungs, emphysema (EM), proteinaceous fluid (PF), hyperplasia of alveolar cells (HY), f) lungs, emphysema (EM), hyperplasia of bronchiolar epithelial cells (HY), sloughed epithelial cells in bronchiolar lumen (arrow)

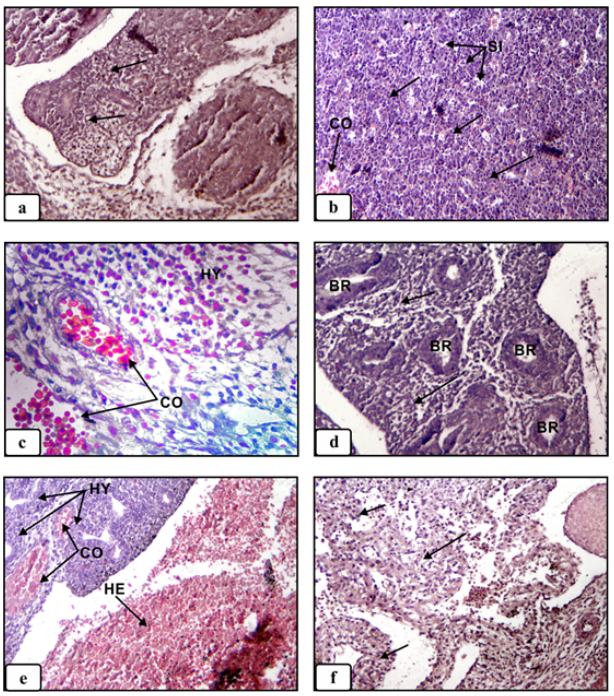

Figure 4: Histological changes in fetuses (NiONPs). A) kidneys, incomplete development of glomeruli and tubules, thickening of interstitium (arrows), b) liver, disarrangement of hepatic cords (arrows), dilatation of sinusoids (SI), congestion (CO), c) Brain, hypercellularity of the brain (HY), congestion (CO), d) Lungs, hyperplasia of bronchiolar epithelium (BR) and alveolar cells (arrows), e) Lungs, hyperplasia of bronchiolar epithelium (HY), congestion (CO), hemothorax (HE), f) heart, fibromuscular tissue (arrows) in place of muscular tissue

placenta and reach fetal units as observed for all the applied models irrespective of the species origin (i.e., rodent, rabbit, or human) or the complexity (i.e., in vitro, ex vivo, or in vivo), and (ii) particle size, particle material, dose, particle dissolution, gestational stage of the model, and surface composition influence maternal- fetal translocation.

The present study showed that administration of NiONPs to pregnant mice induced embryo toxic and teratogenic changes (resorptions, reduced length and weight, intra- abdominal hemorrhages, irregular distribution of fetuses in the uterine horns, and deformed vertebral column and limbs, etc). Some types of nanomaterials are known to be able to cross the placenta into the fetus by passive diffusion or endocytosis, which can trigger fetal inflammation, apoptosis, genotoxicity, cytotoxicity, low weight, reproductive deficiency, nervous damage, and immunodeficiency [14]. A well known fact that particularly in early pregnancy, intrauterine exposure to a toxicant stimulates changes in the embryo and fetus that lead to malformations and stillbirth. Nickel can cross the placenta and has embryotoxic and teratogenic properties [15]. Exposure of zebrafish embryos to nickel nanoparticles produced skeletal muscle abnormalities and defects of the head and jaw cartilage and this change increased with higher nanparticles concentrations [6].

According to results of histopathological examination, NiONPs administered to pregnant mice induced adverse effects in the kidneys, liver, brain, lungs, and heart. Similar results have been obtained by others in non- pregnant mice [5], [16], [17]. It has been stated that NiONPs provoke toxicity through reactive oxygen species generation, which leads to the up- regulation of nuclear factor- kb and Promotes further signaling cascades. NiONPs may induce and provoke oxidative stress and apoptosis [18]. Pathways that are involved in NiONPs toxicity include hypoxia- inducible factor α and mitogen- activated protein kinases. NiONPs have been mentioned to trigger the transcription factors p-p 38, p- JNK, p-ERK ½, Interleukin (IL)- 3, TNF- α, IL- 13, Fas, Cyt c, Bax, Bid protein, caspase- 3, caspase- 8, and caspase- 9 [18].

In this study, NiONPs administered intraperitoneally crossed the placental barrier and induced pathological lesions in the fetus. Microscopically, the lesions were circulatory, degenerative, and hyperplastic. Many types of nanoparticles are well known to cross the placental barrier and to rest in

various organs of fetus. During pregnancy, maternal exposure to nanomaterials led to adverse gestational parameters, neurotoxicity, reproductive toxicity, immunotoxicity, and respiratory toxicity in offspring [19]. Factors that play crucial roles in nanoparticles- induced fetotoxicity include functionalization of the nanoparticles, maternal conditions, and exposure routes. Oxidative stress and inflammation, DNA damage, apoptosis, and autophagy are responsible for the nanoparticles- induced fetotoxicity [19]–[21].

The present study concluded that under the conditions of this study, in which (1, 10 or 20 mg/kg) doses of NiONPs (<50 nm) were administered intraperitoneally daily from the 7th to the 18th gestation day in Balb/c mice, the nanoparticles had adverse effects on the pregnant mice and their embryos. These particles exerted behavioral, abortive, teratogenic, and pathological effects.

Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

References

[1] Kong, Lu., Tong, M., Zhang, T., Wang, D., Hu, K., Lu, W., Wei, C., Liang, G., & Pu, Y. (2014). Nickel nanoparticles exposure and reproductive toxicity in healthy adult rats. *Int. J. Mol. Sci 15*(11): 21253- 21269.

[2] Alsop, D., Lall, S. P., & Wood, C. M. (2014). Reproductive impacts and physiological adaptations of zebrafish to elevated dietary nickel. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 165*, 67-75.

[3] Kong, L., Tang, M., Zhang, T., Wang, D., Hu, K., Lu, W., ... & Pu, Y. (2014). Nickel nanoparticles exposure and reproductive toxicity in healthy adult rats. *International Journal of Molecular Sciences, 15*(11), 21253-21269.

[4] Forgacs, Z., Massányi, P., Lukac, N., & Somosy, Z. (2012). Reproductive toxicology of nickel–review. *Journal of Environmental Science and Health, Part A, 47*(9), 1249-1260.

[5] Magaye, R. R., Yue, X., Zou, B., Shi, H., Yu, H., Liu, K., ... & Zhao, J. (2014). Acute toxicity of nickel nanoparticles in rats after intravenous injection. *International Journal of Nanomedicine,* 1393-1402.

[6] Ispas, C., Andreescu, D., Patel, A., Goia, D. V., Andreescu, S., & Wallace, K. N. (2009). Toxicity and developmental defects of different sizes and shape nickel nanoparticles in zebrafish. *Environmental Science & Technology, 43*(16), 6349-6356.

[7] Singh, M., Verma, Y., & Rana, S. V. (2022). Potential toxicity of nickel nano and microparticles on the reproductive system of female rats: A comparative time-dependent study. *Toxicology and Industrial Health, 38*(4), 234-247.

[8] Biernacki, B, Wlodarczyk, B., & Minta, M. (2000). Effect of sodium valproate on rat embryo development in vitro. *Bulletin-Veterinary Institute in Pulawy, 44*(2), 201-206.

[9] Woods, A. E., Stirling, J. W., & Suvarna, S. K. (2019). *Bancroft's Theory and Practice of Histological Techniques.*

[10] Xiong, P., Huang, X., Ye, N., Lu, Q., Zhang, G., Peng, S., ... & Liu, Y. (2022). Cytotoxicity of metal-based nanoparticles: from mechanisms and methods of evaluation to pathological manifestations. *Advanced Science, 9*(16), 2106049.

[11] Hong, F. & Wang, L. (2018). Nanosized titanium dioxide – induced premature ovarian failure is associated with abnormalities in scrum parameters in female mice. *Int.J. Nanomedicine 13,* 2543-2549.

[12] Wang, Z., & Wang, Z. (2020). Nanoparticles induced embryo–fetal toxicity. *Toxicology and Industrial Health, 36*(3), 181-213.

[13] Bongaerts, E., Nawrot, T. S., Van Pee, T., Ameloot, M., & Bové, H. (2020). Translocation of (ultra) fine particles and nanoparticles across the placenta; a systematic review on the evidence of in vitro, ex vivo, and in vivo studies. *Particle and Fibre Toxicology, 17*, 1-26.

[14] Hou, C. C., & Zhu, J. Q. (2017). Nanoparticles and female reproductive system: how do nanoparticles affect oogenesis and embryonic development. *Oncotarget, 8*(65), 109799-109817.

[15] Genchi, G., Carocci, A., Lauria, G., Sinicropi, M. S., & Catalano, A. (2020). Nickel: Human health and environmental toxicology. *International Journal of Environmental Research and Public Health, 17*(3), 679.

[16] Ajdari, M., & Ziaee Ghahnavieh, M. (2014). Histopathology effects of nickel nanoparticles on lungs, liver, and spleen tissues in male mice. *International Nano Letters, 4*, 1-4.

[17] Wu, Y., & Kong, L. (2020). Advance on toxicity of metal nickel nanoparticles. *Environmental Geochemistry and Health, 42*, 2277-2286.

[18] Iqbal, S., Jabeen, F., Chaudhry, A. S., Shah, M. A., & Batiha, G. E. S. (2021). Toxicity assessment of metallic nickel nanoparticles in various biological models: An interplay of reactive oxygen species, oxidative stress, and apoptosis. *Toxicology and Industrial Health, 37*(10), 635-651.

[19] Teng, C., Jiang, C., Gao, S., Liu, X., & Zhai, S. (2021). Fetotoxicity of nanoparticles: causes and mechanisms. *Nanomaterials, 11*(3), 791.

[20] Abudayyak, M., GÜzel, E., & Özhan, G. (2020). Cytotoxic, genotoxic, and apoptotic effects of nickel oxide nanoparticles in intestinal epithelial cells. *Turkish Journal of Pharmaceutical Sciences, 17*(4), 446-451.

[21] Djebbi, E., Bonnet, D., Pringault, O., Tlili, K., & Yahia, M. N. D. (2021). Effects of nickel oxide nanoparticles on survival, reproduction, and oxidative stress biomarkers in the marine calanoid copepod Centropages ponticus under short-term exposure. *Environmental Science and Pollution Research, 28*, 21978-21990.