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RESEARCH ARTICLE

Investigation on the teratogenic and embryotoxic effects of nanozeolite on chick embryos model

Marziye Hejazy*[1], Masoumeh Moradi[2], Ghasem Akbari[1] , Mohammad Rasoul Amini[3]

[1] Basic Science Department, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

[2] Pathobiologic Department, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

[3] Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

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ABSTRACT

Objective(s): In veterinary medicine, zeolites are used as toxin binder in animal feed, ammonia purification of aquatic pools, animal smell and moisture control. This study investigated on the risk assessment of nano zeolite on development of embryonic chicken models, anomalies, tetratogenic and embryotoxic effects.

Methods: Eggs (120 = n) were accidentally divided into 4 groups. In the experimental groups, 0.3 ml of the solution of nanozeolite (5,50,100 mg/L) injected into egg albumin, The eggs were then incubated for 19 days at 60% humidity and 37.5 ° C. At the end of incubation time, the fetus and organs (liver, heart, brain, spleen) weight and congenital anomalies were investigated.

Results: The administration of nano-zeolite in chick embryos as a model for evaluating human embryonic damage showed teratogenic effects including deformity of legs and wings, liver and heart disformation at the doses of 50,100 mg/L. The embryo were smaller and significant morphological anomalous changes were observed. The comparison between the three experimental groups showed that the dose of 5 mg/L improved viability of chicken and showed increasing the dose of zeolite increases teratogenic effects and increased fetal mortality rate.

Conclusions: The teratogenic effects of nanozeolits on chick embryo should be considered in the risk assessment of nanoparticles on human embryo and fetous.

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INTRODUCTION

Zeolites are crystalline aluminosilicate structures that have a porous structure and have the ability to absorb and release fluids and molecules that have a good diameter. Also, cationic exchanges are not subject to structural changes in the characteristics of zeolites [1]. The special physical and chemic virtues of zeolites have made them appropriate for industry, agriculture, veterinary and environmental applications. Zeolite is used to absorb and remove contaminants in water treatment, reducing and eliminating ammonia and heavy metals in the environment in veterinary medicine zeolites are used as animal feed additive and control of the smell and moisture of pets (animal beds such as dogs and others). [2,3]

* Corresponding Author Email: *mhejazy@ut.ac.ir*

Due to the unique properties of zeolites, nano zeolites have been produced and marketed in recent years. When the size of the zeolite particles goes from micron to the nanoscale, it will significantly improve the efficiency of zeolites in all their applications, but if they the safety issues and their risk assessment is neglected, they can endanger public health.

Pregnancy is a critical period due to the organization of the molecular and cellularunits and because of the high sensitivity of the embryo against environmental toxins. The embryo is sensitive to the effects of xenobiotic due to the lack of development of biological pathways [4]

Chick embryo model is a convenient, simple and accessible model was used in toxicological

studies to evaluate the toxic and teratogenic effects of the substances. Furthermore, the independence of the fetus from the mother and the rate of growth of the embryos can allow the immediate evaluation of the direct nanomaterials effects on the fetus in a short time [5,6]
The goal of this study was to investigate the effects of embryotoxic and teratogenic nanozeolites on chick embryos in ovo environment.

MATERIAL AND METHODS

Nano particles synthesis and characterization

The nano zeolite particles with average size of 80 to 100 nanometers were hydrothermally synthesized from local annealed clinoptilolite after procedure consisting of grinding, drying, and washing. NaOH, Clinoptilolite, distilled water and Sodium aluminate were mixed for six hours (aging process). The prepared gel was sonicated for six hours, transferred to a stainless steel Teflon-lined autoclave then heated at pressure in an air oven at 353 K.

After a proper time (the pH reached to 7). The provided suspension was centrifuged and the final product was dried to preparation of nano-zeolites. The products were characterized with SEM and XRD.[7]

Embryos treatment with nnozeolite

Broiler eggs (n=120) was purchased from the Ross breeder Incubation Company. The eggs were incubated at 12 ° C for four days. After four days, the eggs were weighed and divided into 4 groups randomly (one control group and three experimental groups n=30). In the control group, only 0.3 ml of physiologic serum was injected. In the test groups, 0.3 ml of nanozeolite solution (5, 50, 100 mg / L) was injected into the egg albumin (2/3 of egg height at rounded end) using a sterile 20 milliliter syringe at sterile condition. The injection site was sterilized with alcohol and covered with paraffin. The eggs were incubated in 60% humidity at 37.5 ° C and displaced once a day.

Sampling and gross pathology examination

At the end of 19th day of incubations, live embryos were sacrificed and the embryos and organs (heart, brain, spleen, liver) were weighed and congenital anomalies and the teratogenic effects of nanozeolite and was evaluated.

RESULTS AND DISCUSSION

Nanozeolite characterization

Scanning electron microscope (SEM) images of nanozeolite is provides in Fig. 1. The x-ray diffraction (XRD) patterns showed reflections corresponding to the 4A zeolite; no other structures were present Fig. 2.

Evaluation of embryogenic defects and alterations

Administration of nano-zeolite in development stage of chick embryos as a model for evaluating human embryonic damage has shown that an increase in the dose of nano-zeolite is associated

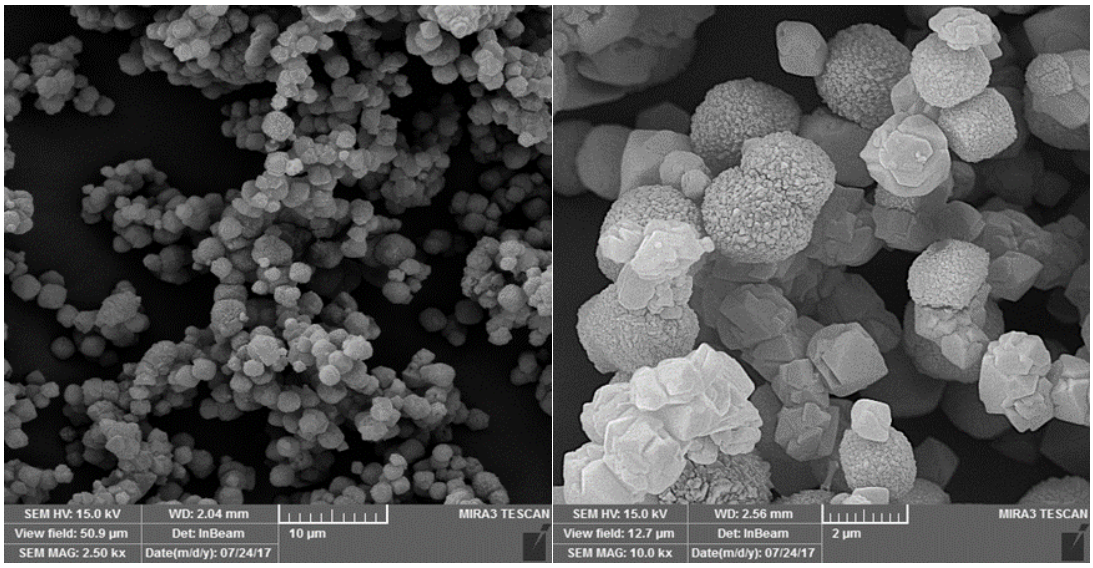

Fig. 1. a represent scanning electron microscope (SEM) images of nanozeolite.

with increase in the mortality rate of embryos in different stages of growth. 19-day evaluations showed that the administration of nanozolite at doses of 5 and 50 mg / L resulted in increased viability and reduced side effects and increased the fetal mortality rate by 66% at a dose of 100 mg / l. The results showed that nanozeolite decreased significantly the spleen weight dose dependently

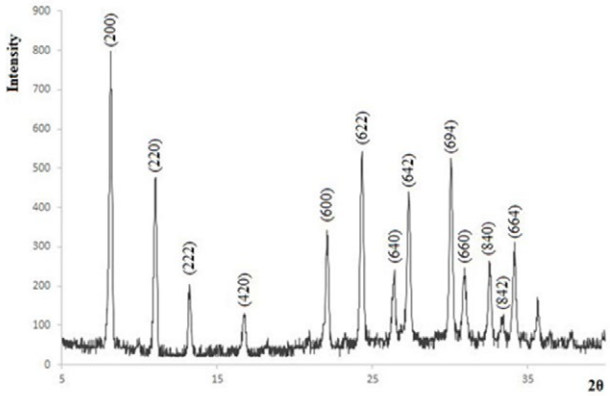

Fig. 2. X-Ray Diffraction (XRD) patterns of zeolite

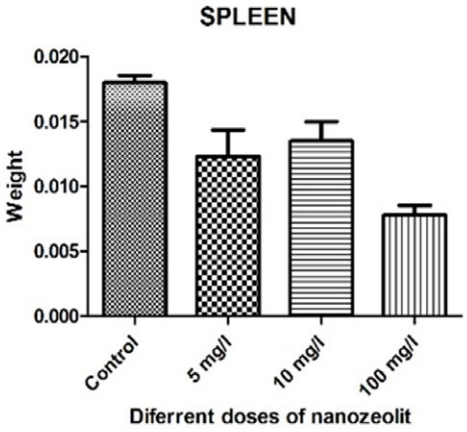

Fig. 3. showed that nanozeolite decreased significantly the spleen weight

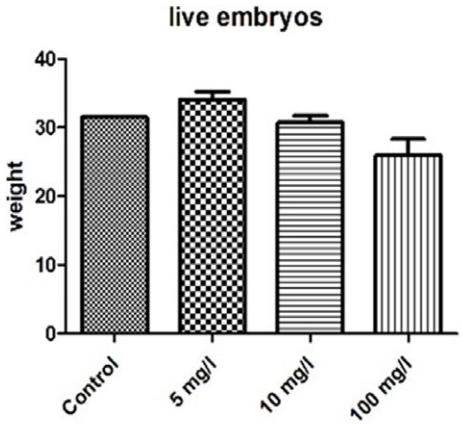

Fig. 5. decreased the liver weight at the dose of 5mg/L

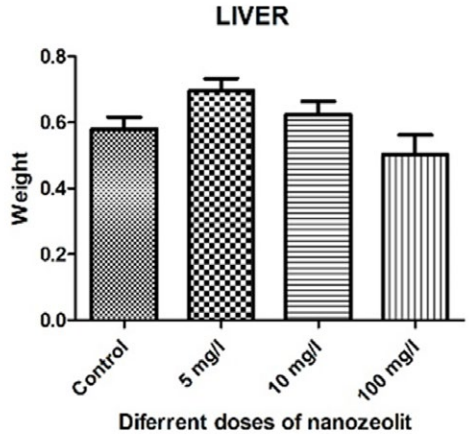

Fig. 4. increased the embryos weight significantly at the dose of 5mg/L

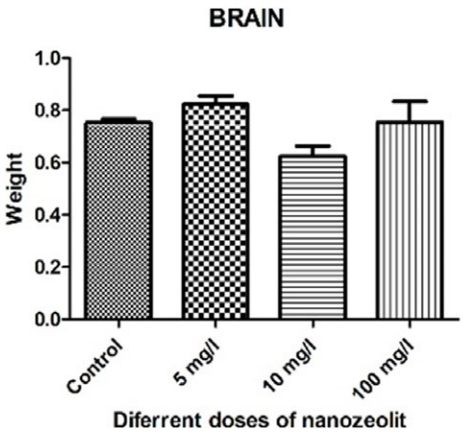

Fig. 6. elevated the brain weight at the dose of 5 mg/L

Fig. 7. Control group

Fig. 8. large yolk sac which not absorbed by the chick

Fig. 9. Teratogenic effects were including deformity of legs and wings

(P<0.05) (Fig. 3). Nanozeolite also increased the embryos weight significantly at the dose of 5mg/L but at the higher doses the weight decreased significantly (P<0.05). (Fig. 4). It also decreased the liver weight at the dose of 5mg/L, but at the higher doses the liver weight decreased. (Fig. 5). Nanozeolite have elevated the brain weight at the dose of 5 mg/L (P<0.05) (Fig. 6).

In the administration of nano-zeolite, the embryo had a smaller sizes and significant morphological and anomalous changes, increase in embryogenic defects and teratogenic effect on embryos in different stages of growth special at higher doses was observed (Fig. 7).

19-day evaluations showed that the administration of nanozolite at doses of 5 and 50 mg /L resulted in rigid large yolk sac which not absorbed by the chick (Fig. 8). Teratogenic effects were including deformity of legs and wings and liver and heart disformtion (Fig. 9). The comparison between the three experimental groups shows that increasing the dose of zeolite increases toxic effects on the development of the embryos.

Zebrafish embryos exposure with Ag nanoparticles delayed hatching and causes mortality concentration-dependently [8,9,10]

Abnormality changes included spinal cord truncation and flexure, finfold deformities, defective notochord, cardiac and eyes abnormalities [8, 11, 10]

Congential defects included nondepleted and opaque yolk, small jaw and head and circulatory malformations [12]

Silver/ palladium or silver/ copper nano particles did not influence embryonic chick development [13,14]They did not have any negative effects on, growth, developmental or morphological embryonic survival of chicken embryos.

Silver nanoparticle affected the metabolism of layer but it have no effect on broiler embryos weighting and development [15]

Chicken embryos exposure to cadmium sulfide NP resulted in increased developmental anomalies dose-dependently [16]

CONCLUSIONS

Nanozeolite particles are unique compounds that are used to produce medical and veterinary products. But their safety and their interactions in the body is still under discussion. The study of nanozeolite fetal toxicity is very important because of the possibility of their passage from the dams. This study suggests that increasing the dose of zeolite showed toxic effects on the development of the fetus and increased fetal mortality rate and tertogenic effects. The teratogenic effects of nanozeolits on chick embryo should be considered in the risk assessment of nanoparticles on human embryo and fetous.

The penetration of nanoparticles into different tissues and organs and blood flow and their effects on their longevity or their final effects must be studied in order to alleviate their use and minimize adverse effects.

More studies are needed to develop appropriate strategies to protect against the embryotoxic effects of nanoparticles. So far, a limited number of tests have been carried out in different fetal models, so the possibility of comparisons is limited, and studies are required to address the toxic effects of the certain species. However, animal-dependent differences in the toxicity of similar nano-particles should mentined in different species. In this case, chick embryos appear to be more resistant to exposure to nanoparticles. But dose dependent toxicity and tetratogenicity was observed in chicks. Several questions have been raised about the safety of nanoparticles that have become unresponsive, which should be addressed promptly in light of the increasing use of nanoparticles in medicine and veterinary medicine.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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