Investigation of the Zinc Oxide Nanoparticles Effect on Testosterone, Cholesterol and Cortisol in Rats

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Abstract

Nowadays nanoparticles have widespread application in various industries because of their special and unique features. There are many studies in side effects of nanomaterial but these studies used from high doses and specially ZnO nanoparticles in low dose was less researched. This study done by 36 Wistar rats with every other day injection of different doses of ZnO nanoparticles intraperitoneally (5, 10, 20 and 40 mg / kg). After a 21-day period, the rats were bled and whole blood hematocrit and lymphocyte numbers and serum levels of cholesterol, cortisol and testosterone hormone were measured. The results showed a significant decrease of cortisol level and significant increase of testosterone and cholesterol level of blood serum in rats that injected by ZnO nanoparticles rather than controls. Number of lymphocyte in treated rats reduced and hematocrit showed an irregular variation. Therefore all of results confirmed exposing to ZnO nanoparticles damaged to public health and reduced fertility potential.

Keywords: Cholesterol, cortisol, nano zinc oxide, testosterone, Wistar rats.

Introduction

Nanoparticles have unique physical and chemical properties of size, shape and high surface to volume ratio that these properties have appropriated for biological, medical widespread applications. Unfortunately the dose of nanoparticles had been used in vitro and in vivo researches was high dose. Although in some cases the lowest dose was used, but it was so low and negligible¹.

In general, nanotechnology is production of new materials, instruments and systems and taking their control in molecular and atomic level and using of their properties in nanoscale². Nowadays it is reported increasing concerning about the effects of these substances on human health and environment because, some nanoparticles produce reactive Oxygen (ROS)³. It also causes toxicity in lab environment. Nano particles can pass through cell membrane easily. They also pass through blood-brain barrier and blood-testes barrier⁵. This proved that liver parenchymal cells have a major role in the remove of nanoparticles from blood and detoxification⁶.

ZnOnano particles have widespread application such as skin cream to prevent sunburn (sunscreen creams), food additives, pigments, cell imaging, photodynamic therapy, biosensor, UV detector, resin production, drug carries, catalysts and electronic materials⁷,⁸. In recent years there were many researches on benefits, risks and toxicity of nanoparticles. Recent studies showed that ZnOnanoparticles could be used in the medicine for treatment of diabetes, cancer and autoimmune diseases⁹-¹¹.

ZnOnanoparticles have negative effects on bacterial growth like staphylococcus, streptococcus and E.coli so they can prevent spreading of epidemic diseases as an etiological agent¹²-¹⁴.

However, toxicological studies have shown that ZnOnanoparticles could be harmful to human and other species¹⁵. Han et al. reported neurotoxicity in rats after intraperitoneal administration of ZnOnanoparticles¹⁶. Many studies have reported the cytoxic and genotoxic effects of ZnOnanoparticles in various mammalian cell lines¹⁷-¹⁹. ZnO particles can cause immune toxicity. Monocytes are more sensitive than lymphocytes²⁰.

There are many studies in side effects of nanomaterial but these studies used from high doses and specially ZnOnanoparticles in low dose was less researched. In the present study we investigate the effect of the different doses of ZnOnanoparticles on testosterone, cholesterol and cortisol in rats.

Material and Methods

This experimental study was performed on 36 Wistar rats. After preparing these animals from the Isfahan University of Medical
Sciences, they were kept a month in order to prepare in Payam Noor University of Isfahan. Testing carried out at temperature of 20-25 centigrade degree that day duration was 12 hours and 12 hours dark lighting. Experimental animals were average weigh 300+30 gr and they were divided into six groups. First control group feed by usual water and food. Second control group injected by 1 ml distilled water every other day intraperitoneally for equivalency of shock that obtained by treatment as placebo. Other groups from 3rd to 6th injected by 1 ml ZnO nanoparticles in 5, 10, 20 and 40 mg/kg doses, injection repeated every other day intraperitoneally. This continued until 21 day. After 21 days and 10 times injection, one day after last injection, blood sample was prepared as follow. The animal was anesthetized with chloroform in special glass container of desiccator and then blood sample is prepared from neck vain. After extraction of blood, it is slowly poured into a clean test tube and about 15 to 20 minutes keep immobile in the laboratory temperature for clotting and separating the serum then samples were centrifuged at 3000rpm for 30 minutes, after this time remove the tubes and the clot by Smplr, serum is poured into a container cap and stored in freezer then used for cortisol and testosterone measurement (using the chemiluminescence technique) and cholesterol level of serum measured also with using enzymatic method (point to point). We also measured number of lymphocyte and hematocrit by using of whole blood.

The results analyzed based on the statistical program SPSS and analyzed by ANOVA and Tukey test was the difference in the level P <0.05 was considered significant.

**Results and Discussion**

Statistical studies and comparison of testosterone, cortisol and cholesterol hormone average concentrations in animals that threatened by ZnO nanoparticles and controls were done. Results with statistical analysis are shown in figure. Asterisks* indicate significant differences at P <0.05 for each test group rather than the control group. Results showed injection of ZnO nanoparticles with different doses have a significant effect on cortisol concentration (figure 1). Cortisol level showed a slight increase with 5 mg dose but 10, 20, 40 doses are decreased that this reducing is significant by 40 mg dose.

Testosterone hormone level in groups that received 5, 20, 10 mg/kg doses of nanoparticles don't show significant increasing rather than controls but there is a significant variation between group that treated by high dose of ZnO nanoparticles and control group (figure 2).

Results showed a significant increase in cholesterol level in high concentration (40mg/kg) (figure 3). Figure 3 showed cholesterol level of treated and control groups.

In this study we indicate that presence of ZnO nanoparticles decreased the number of lymphocyte in rats’ blood and this reduction was dose dependent, so high dose treated rats showed more reduction (figure 4).

Figure 5 showed irregular variation of hematocrit in treated and control rats. Lower dose of ZnO nanoparticles causes enhancement in hematocrit percentage and high dose cause significantly reduction of hematocrit. In recent years, there have been an increasing number of studies on toxicity of ZnO nanoparticles and they have been shown to affect many different cell types and animal systems. Generally, the toxicity of ZnO nanoparticles is related to their small size, concentration, bio distribution and high specific surface area.$^{21,22}$

![Cortisol concentration of treated and control groups. Result showed high dose of ZnO nanoparticles causes significantly reduction of cortisol and its effect is dose dependent, Asterisk symbol showed significant changes (P<0.05)](image-url)
Figure-2
Testosterone level of rats’ serum showed increasing of this hormone concentration by ZnO nanoparticles exposing. Asterisk symbols showed significant changes by P<0.05

Figure-3
Serum cholesterol measurement showed enhancement of cholesterol level in presence of ZnO nanoparticles. Asterisk symbol showed significant variation (P<0.05)
Numbering of lymphocyte in treated and control rats showed significantly reduction of lymphocyte numbers especially in high dose of nanoparticles. Asterisk symbols showed significant changes by $P<0.05$.

Hematocrit percentage showed injection of lower nanoparticles dose increased hematocrit but high dose significantly decreased it. Asterisk symbol showed significant changes ($P<0.05$).
Testicular androgens are produced by interstitial tissue of Leydig cells. Instant precursor of gonadal steroids is such as Adrenal steroids, cholesterol. Results indicate ZnO nanoparticles with mentioned doses have different effects on testosterone, cortisol hormones and cholesterol. Because of cholesterol is precursor of testosterone, by increasing cholesterol, this study shows significant decrease in cortisol that has steroid structure basically. Some studies indicate nanoparticles can be important in protein expression (Steroidogenic-Acute regulatory). This protein in the transfer of cholesterol to mitochondrial membrane and steroid increasing. Nano ZnO reduces serum cortisol due to destructive effect of nanoparticles on adrenal glands.

A study has done by Roshanayi and associates to silver nanoparticles are caused reduced cortisol and increased testosterone. Because of influence on adrenocorticotropic is secreted by pituitary that can be related to penetration of ZnOnanoparticles from blood-brain barrier and perhaps it is effect antioxidant activity of Zinc on cortisol secretion.

In a research has been proven inhibitory effect of Zinc on cortisol secretion. Relation between cholesterol and testosterone in negative because testosterone enhanced and cortisol reduced this relationship affected reproduction or infertility potential such recent research indicate.

**Conclusion**

Results showed other blood factors such as number of lymphocyte decreased that may be cause susceptibility to infectious disease. Hematocrit showed variable changes against the nanoparticles injection, it increased in lower dose but when higher dose (40 mg/kg) administrated hematocrit significantly reduced. It showed toxicity of nanoparticles is extremely dose dependent. Nanoparticles also cause significant and dose dependent changes in testosterone and cortisol levels of blood that may be affected fertility potential of rats.

**References**

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