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The Impact of Zinc Oxide Nanoparticle on LH, FSH, and Testosterone Hormones in Mature Male NMRI Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Nanoparticles are widely applied in all aspects of modern life because of their unique features such as small size and high surface area. Several types of research have been carried out to discover the feasible detrimental impacts of Nano-particles on human reproduction. The purpose of this study was to examine the impact of zinc oxide nanoparticles in mature male rats through examining LH, FSH, and testosterone sex hormones. Therefore, 30 Naked Mole-*Rat* Initiative (NMRI) rats were divided into 5 groups. Different doses of zinc oxide nanoparticles (250, 500 and 700 $mg.kg^{-1}$) were intra peritoneally injected to animals only once. Then, the serum level of luteinizing hormone (LH), Follicle Stimulating Hormone (FSH), and testosterone hormones were measured using Enzyme-Linked Immunosorbent Assay (ELISA) method after 21 days. The results were analyzed by ANOVA and Tukey tests. The results indicated that zinc oxide nanoparticles doses caused a significant increase in FSH and testosterone level of blood (Respectively) in 250 and 700$mg.kg^{-1}$ in comparison with the control group. Moreover, this research illustrated that zinc oxide nanoparticle can cause a dose-related increase in Testosterone and FSH hormones levels while causing no significant change in LH hormone level.

Keywords: Zinc oxide nanoparticles; hormone; testosterone; LH; FSH; rats.

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1. INTRODUCTION

Nanotechnology is the functional system engineering at the molecular scale, which recently became progressively important in fields including engineering, construction, agriculture, microelectronics, and health care. In recent years, the use of nanotechnology in the field of medicine and health care has attracted much attention. Today, there are many treatments that are very time consuming and expensive. However, quicker and much cheaper treatments can be developed using nanotechnology [1]. The Nanoscale instruments could be 100 to 10,000 times smaller than human cells, but they are similar in size to large biomolecules such as receptors and enzymes. Nanoscale instruments smaller than 50nm can easily pass in mast cells, and those smaller than 20nm can pass through blood vessels and enter the bloodstream [2]. New nanoparticles and nanomaterials are considered, which have been very much considered, have a major impact on different areas. High-performance metals are made on a Nanoscale with unique properties so that traditional synthesis and industrial methods cannot create. In the future, nanoparticles should act as drug-targeting systems (DTS) and drug-delivery (DDS). Nanoparticles are not detected by human blood due to their small size, thus, they transmit through cell membranes and are able to pass the blood-brain barrier [3]. Zinc oxide nanoparticles (ZnO NPs) is a metal oxide nanoparticle that, has been considered in biology and medicine rather than its technological application [4]. Zinc oxide nanoparticles are commonly made in terms of engineering, which are used on a large scale. The most common use of zinc oxide powder is the use of oxide reactivity as a precursor to other zinc compounds [5]. The use of the high refractive index, high thermal conductivity, and binding properties of ZnO are the applications in material science. Possible exposure to ZnO NPs could occur in the industrial fields through daily consumer products. ZnO NPs are added to various materials and products, for example, plastics, glass, ceramics, rubber, cement, paints, lubricants, ointments, sealants, adhesives, batteries, pigments, ferrites, and fire retardants. Furthermore, ZnO nanometals have ultraviolet (UV)-shielding, deodorizing impacts, antibacterial properties, impact and heat and UV light resistance, which could afford a great potential for its widespread use in many fields including cosmetics and sunscreens [6], fungicides in agriculture [9], food additives and additives in packing [7,8], and biomedical uses such as anticancer drugs [10,11]. Nanoparticles are highly reactive due to their small size as well as high surface area. This is one of the important reasons for their toxic impacts [12]. Some reports state that ZnO NPs inhalation cause cell toxicity and inflammation. Some other types of research have also demonstrated that kidneys and spleen are the target organs of nanoparticles after oral administration, [13]. However, their risk for humans and mechanisms of toxicity are not well defined.

Zinc is essential for optimal performance and efficiency in the male reproductive system and helps to stabilize the nuclear chromatin of spermatozoa and cell membrane in seminal fluid [14]. Other roles of zinc in male reproduction may be a regulatory role incapacitation and acrosome reaction process [15] that can protect the testis against degenerative changes [16]. Zinc (Zn) has many very important biological reactions with hormones [17]. A report showed that zinc has a role in the storage, production, and secretion of hormones to increase the impassiveness of end-organ responsiveness and receptor sites [18]. One of the most significant impacts of Zn deficiency is on hormone production and secretion of testosterone, spermatogenesis, insulin and adrenal corticosteroids. Moreover, the development of the primary and secondary sex organs in the male and all the reproductive process phases in the female can be adversely influenced by Zn deficiency [19]. The most common compound of Zn is ZnO [20]. Since it has the highest zinc concentration [21], and it is high absorption in the body and by Gastrointestinal better tolerated makes it preferable [22]. Since ZnO NPs is a significant unintended human exposures source, more knowledge about other properties of zinc oxide, such as its impact on sex hormones, is very important and necessary. Therefore, the purpose of this research was to evaluate the impacts of different doses of zinc oxide nanoparticle on LH, FSH, and testosterone (TSS) sex hormones level in mature male rats' blood.

2. MATERIALS AND METHODS

2.1 Experimental Design

In this experimental research, 30 mature male NMRI rats with a weight range of 28-32 g were purchased from Pasteur Institute of Iran. They were kept in Plexiglas cages in an animal room at 23 ± 2°C under a 12h light.12h^{-1} dark cycle

with free access to food and water. The rats were divided into 5 groups (6 rats in each group) as follows:

First Group (control): No treatment
The second group (sham): Rats received distilled water.
Third, fourth, and fifth groups (Experimental): Zinc oxide nanoparticles were injected to the rats in doses of 250, 500, and 700 mg.kg^{-1}, respectively.

All injection was done in the form of intra-peritoneal only once, treatment volume was 1 ml. The rats were anaesthetized by diethyl ether one week after injection, and then, an autopsy was done. 1 mL blood sample was taken from the left ventricle of the heart of each rat with a 2ml syring. The blood samples were poured into a 1.5 mL micro tube and vertically placed in a laboratory environment for 0.5-1 hours. Then, the blood samples were centrifuged at 2500 rpm for 5 minutes. Afterwards, the serum was isolated by the sampler from the clot and stored in a freezer at -20°C to determine serum levels of testosterone, FSH, and LH. The ELISA method was applied to determine the level of sex hormones.

In this research, the white powder of zinc oxide nanoparticles (Pars Lima Company, the Particle size of 20 nm and purity of 90%) has been used that is very toxic to aquatic organisms. In addition, distilled water was used as the solvent.

2.2 Statistical Analysis

The collected data were analyzed through SPSS software (version 21) along with ANOVA and Tukey test. The statistical criterion was considered as P <0.05.

3. RESULTS AND DISCUSSION

According to the results, the impact of different doses of Zinc oxide nanoparticle (250, 500, and 700 mg.kg^{-1}) on experimental rats groups showed a significant increase ($p<0.01$**) in FSH hormone level in animals that treated by 250mg.kg^{-1} dose of Zinc oxide nanoparticle in comparison with the control group. However, other doses (500 and 700 mg.kg^{-1}) did not show a significant impact on FSH hormone level (Fig. 1). As observed in Fig. 2, the intraperitoneal injection of zinc oxide nanoparticles at different doses of 250, 500, and 700 mg.kg^{-1} did not significantly increase the level of LH hormone in comparison with the control group. Rats that received 250 and 500 mg.kg^{-1} doses of zinc oxide nanoparticle did not indicate a significant difference in testosterone hormone level compared with the control group. However, the injection of a high dose of zinc oxide nanoparticle (700 mg.kg^{-1}) resulted in a significant increase in Testosterone hormone level compared with the control group (Fig. 3).

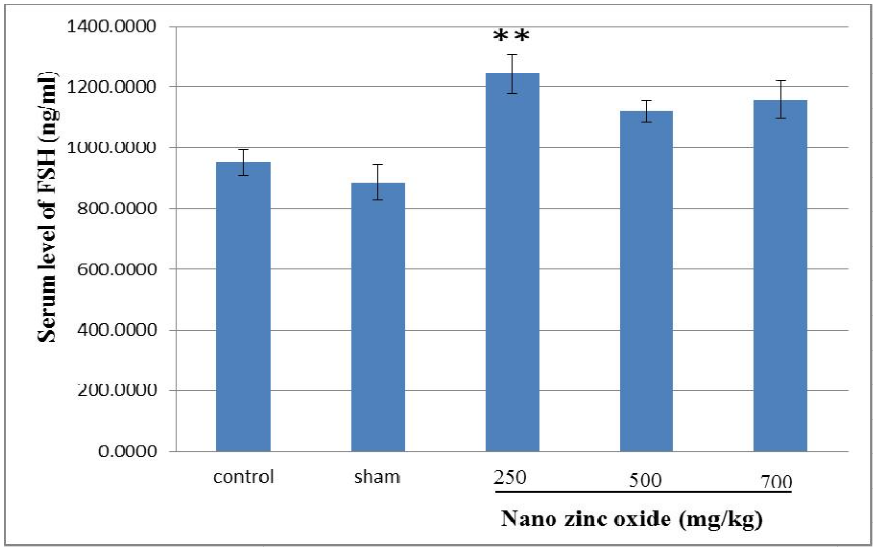

Fig. 1. The impact of different doses of zinc oxide nanoparticle (250, 500, 700 mg.kg-1) on blood FSH level

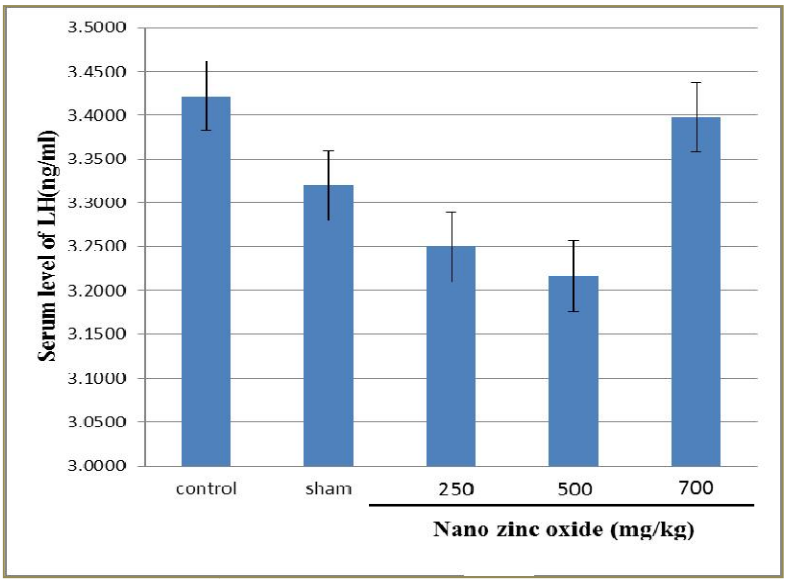

Fig. 2. The impact of different doses of zinc oxide nanoparticle (250, 500, 700 mg.kg-1) on blood LH level

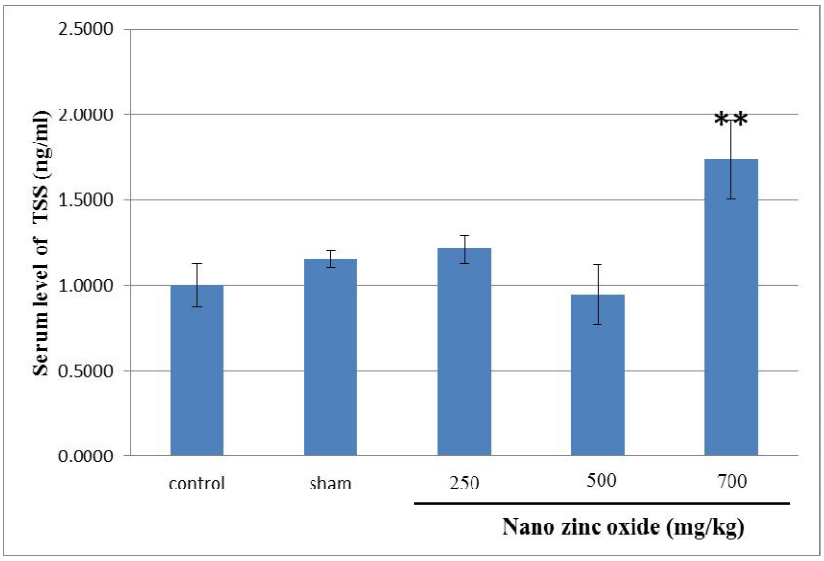

Fig. 3. The impact of different doses of zinc oxide nanoparticle (250, 500, 700 mg.kg-1) on blood Testosterone (TSS) level

The treatment of the experimental groups was different doses of zinc oxide nanoparticle. Sham groups received distilled water only once. Injection of 250 $mg.kg^{-1}$ zinc oxide nanoparticle significantly increased the FSH hormone serum level. The results of ANOVA, **P<0.01 test showed the difference from the control group.

Different doses of zinc oxide nanoparticles in animals that received did not show a significant impact on the level of LH hormone compared with the control group. The results were achieved by ANOVA test.

The experimental group that was treated by 700 $mg.kg^{-1}$ zinc oxide nanoparticles showed a significant increase in the serum level of Testosterone hormone compared with the control group. The ANOVA, **P<0.01 results showed a significant difference in the control group. Zinc

oxide nanoparticle has recently attracted the attention of researchers in the field of animal studies [23]. Nanoparticles have different shapes of new materials with outstanding biological properties and low toxicity, which seem to have a very high potential in crossing physiological barriers and access to specific target tissue [24]. The present research revealed that intra-peritoneal injection of high-dose zinc oxide nanoparticle (700 $mg.kg^{-1}$) significantly increased the testosterone hormone level compared with the control group.

Some studies showed that nanoparticles could affect the Steroidogenic Acute Regulatory protein (STAR) expression [25]. This kind of protein (transport protein) transports cholesterol into the inner mitochondrial membrane. It also increases steroid hormones production [26]. Possibly zinc oxide nano-particles transport cholesterol into the inner mitochondrial membrane through increasing the StAR protein expression and eventually convert the cholesterol to pregnenolone. Therefore, testosterone levels increase. Zinc is a cofactor for more than 200 enzymes, with multiple important functions, the most important of which is participation in the system of antioxidant defence [27]. The mechanism applied by zinc in its antioxidant activities is not well known. Nevertheless, results proposed that zinc increases the synthesis of metallothionein, which is a cystein-rich protein that acts as a free radical scavenger [28] and hence, it enhances the fertility potential [29]. Cells are unable to form sex steroids in zinc deficiency, which leads to spermatogenesis arrest and fertility impairment [30].

Hambidge et al. [31] expressed that zinc is essential in producing many sex hormones such as testosterone hormone [31]. ZnO is used as a zinc source in the nutrition industry [13]. Therefore, it can be one of the factors associated with increased testosterone levels in the present study.

In this study, it was found that rats that were treated with low-dose zinc oxide nanoparticle ($250mg.kg^{-1}$) only once, indicated an increase in the dose of FSH hormone in comparison with the control group.

The change in FSH hormone level can occur because of passing nanoparticle from the blood-brain barrier given the fact that FSH and LH are pituitary hormones [32]. The other result was that nanoparticles could be altered in endocrine glands function [33], which has led to increased FSH hormone from the pituitary gland in the case of zinc oxide nanoparticles. Some types of research have indicated that catecholamine has an inhibitory impact on the GnRH release. In addition, it seems that GABA inhibits the release of FSH and LH [34]. There is a dopamine neural pathway from black body to nucleus caudatus and putamen, as well as neural pathway of gamma-aminobutyric from 2 nucleus to black body. Some studies revealed that the increased levels of zinc in the black body causes destruction and decreased dopamine release in the black body cell. The other side, the GABA nerve pathways are directly inhibited by the increased zinc [35]. Since the zinc oxide is the most common combination of zinc [20], Zinc oxide nanoparticle is likely to reduce the level of dopamine increases FSH hormone level, although no impact on LH level. In addition, some studies show that dopamine in middle prominence in the negative feedback of testosterone may be involved in the release of GnRH [34]. In other words, the hypothalamus can be affected by increasing the testosterone in the form of negative feedback and decrease in LHRH. Consequently, this decreases LH secretion [25]. The results of the present research indicated that LH hormone level is not affected by increasing the testosterone impact. The LH hormone does not change even with the increase in testosterone probably due to the mentioned reasons. Espanani et al. [36] examined the impacts of the Zinc Oxide Nanoparticle on Sex Hormones. The experimental rats were injected by 1 ml zinc oxide nanoparticle in 5, 10, 20 and 40 $mg.kg^{-1}$ doses, which repeated 21 days intraperitoneally. They concluded that groups receiving a high dose of Zinc oxide nanoparticle ($40mg.kg^{-1}$) indicated a reduction in FSH concentration compared with the controls [36], which is not consistent with our results. However, similar to the results of the present study, injection of zinc oxide nanoparticles did not have a significant impact on LH hormone level. High doses of zinc oxide nano significantly increased the testosterone level. Therefore, it can be concluded that the injection of $250mg.kg^{-1}$ has a better impact on FSH hormone only once rather than lower doses ($40\ mg.kg^{-1}$) with daily repeated injection.

4. CONCLUSION

Evaluation of the impact of different Nano-particles impact such as gold, silver, and Tio_2 on

sex hormones showed that these Nano-particles have a negative impact on testosterone hormone, which increases the LH hormone level by negative feedback. However, these nanoparticles did not have a significant impact on the level of FSH hormone [37-39]. Nevertheless, the results of the present research illustrated that zinc oxide nanoparticle increased the Testosterone and FSH hormones levels depended on their dose compared with the control group while there is no significant change in the level of LH hormone. It can be concluded that zinc oxide nanoparticle in the relevant doses do not negatively affect sex hormones. Accordingly, it can be concluded that Zinc oxide nanoparticle can reduce the problems of male sex hormones decline.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard Animal committee ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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