Cihan University-Erbil Scientific Journal (CUESJ)

Research Article

Investigation of the Zinc Oxide Nanoparticles Effect on Thyroid and Testosterone Hormones in Male Rats

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ABSTRACT

Exposure to zinc oxide nanoparticles (ZnO NPs) has been increasing steadily, causing more attention being paid to their potential toxicity, including cytotoxicity and genotoxicity. Hence, this study aimed to investigate the effect of ZnO NPs on thyroid hormone triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) as well as testosterone hormone in male adult rats. A total of 54 Sprague-Dawley albino adult male rats were divided into nine groups each of six rats, daily treated intraperitoneal with ZnO NPs two different doses (30 and 60) mg/kg in three different periods of time (7, 14, and 28) days, as following: Control groups (Groups 1, 2, and 3): Respectively received intraperitoneal injection with distilled water for 7, 14, and 28 days, experimental groups (Groups 4, 5, and 6): They were rats, respectively, received intraperitoneal dose (60 mg/kg) of ZnO NPs for (7, 14, and 28) days, and group (7, 8, and 9) experimental groups were rats, respectively, received intraperitoneal dose (30 mg/kg) of ZnO NPs for (7, 14, and 28) days. Data showed high significant decrease ($P < 0.01$) in level of T3, T4, TSH, and level of testosterone also decrease at high and low dose for 7, 14, and 28 days.

Keywords: T3, T4, testosterone, thyroid-stimulating hormone, zinc oxide nanoparticles

INTRODUCTION

Zinc oxide nanoparticles (ZnO NPs) are inorganic compounds, called multifunctional material due to unique chemical and physical properties.[1] Zinc is an essential metal it is an activator for more than 300 enzymes in the body.[2] ZnO NPs are one of most widely used in cosmetics and sunscreen because of their efficient UV absorption properties, they are used due to the antimicrobial properties in food packaging.[3] They are also being explored for their potential use as fungicides in agriculture.[4] As well ZnO nanoparticles are used in anticancer drugs and in biomedical applications.[5] Commonly considered to be a material with low toxicity because the ZnO is an essential trace element in human body and is existing in food or added as nutritional complement, so zinc attracts little care during assessment of toxicity of NPs.[6]

ZnO NPs can induce the formation of reactive oxygen species (ROS) that disrupt intracellular metabolic activities and the antioxidant system; these alterations permit generated ROS to interact with and damage DNA, lipids, carbohydrates, and proteins.[7] Thyroid gland is one of major endocrine glands of the body responsible of creating the hormones, thyroxin, and triiodothyronine which are essential for the proper organism development in particular for the nervous system and heart, normal growth, and skeletal maturation.[8] It is considered one of the main regulators of biological processes, during development, and childhood.[9] Decrease in the production of thyroid hormones means hypothyroidism.[10] Many evidence suggesting the role of zinc in the formation and function of thyroid hormones.[11] ZnO NPs cause higher levels of oxidative stress, resulting in inflammation, and cell toxicity.[12] Man testis has two main functions, the production of testosterone and the production of male germ cells, maintenance of spermatogenesis process needs testosterone, the androgen production is regulated by luteinizing hormone, while follicle-stimulating hormone is critical for initiation and maintenance of spermatogenesis.[13,14] Testosterone is the main androgenic hormone in males, it is largely produced by the Leydig cells of the testes, ZnO NPs were internalized by Sertoli cells and Leydig cells resulted in cytotoxicity in a time and dose-dependent manner through the induction of apoptosis, caused by increase in ROS related with loss of

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Received: Apr 25, 2019
Accepted: Apr 28, 2019
Published: Jan 20, 2020

DOI: 10.24086/cuesj.v4n1y2020.pp26-31

mitochondrial membrane potential, so injection of ZnO NPs produced structural alterations in the seminiferous epithelium and sperm abnormalities in male rats.[15]

MATERIALS AND METHODS

Adult Male Sprague-Dawley albino rats, age about 2.5–3 months, average bodyweight 200–225 g. They were obtained from the National Center for Drug Control and Research/ministry of health, then transferred to the animal house of the college of science, Mustansyriah University. All animals were allowed to acclimatize to the laboratory conditions for 7 days before starting the study. They were kept in clean separated plastic cages with metal network cover under climate-controlled condition of the animal house with 22–25 temperature, 60% humidity, 12 h light, and darkness period and allowed free access to food *ad libitum* and water. This study was conducted after obtaining the approval from the ethical consideration to deal with experimental animals that is the Ethical Committee of the College of Science, Mustansiriyah University and all ethical behavior with the laboratory animals was taken care and has priority in our work in accordance with the globally adopted guidelines of the animal care and experiments.

Preparation of ZnO NPs Solution

ZnO NPs used in this study was obtained from sky spring nanomaterials, they were in white to light yellow colored powder with 99.8% purity, particle size was 10–30 nm in diameter. The stock suspension was prepared by dissolving 1 g of powder ZnO in 10 ml of distilled water and then mixed by vortex for 10 min to prevent agglomeration, then distributed in to following groups:

Group of 60 mg/kg of ZnO NPs (high dose) 120 μl of stock +880 μl of distal water.
Group of 30 mg/kg of ZnO NPs (low dose) 60 μl of stock +940 μl of distal water.

Experimental Design

To study the effect of ZnO NPs, animals were divided into nine groups with six rats each as follows:

- Groups 1, 2, and 3 (control group), respectively, received intraperitoneal injection of distilled water for (7, 14, 28) days.
- Groups 4, 5, and 6 (the experimental groups); rats, respectively, received intraperitoneal dose (60 mg/kg) of ZnO NPs for 7, 14, and 28 days.
- Groups 7, 8, and 9: (The experimental groups); rats, respectively, received intraperitoneal dose (30 mg/kg) of ZnO NPs for 7, 14, and 28 days.

Measurement of the Levels of Hormones Concentration

It was represented by the enzyme immunoassay tests (TOSOH) for the quantitative determination of concentrations of thyroid gland hormones T3 according to Braverman *et al*.,[16] T4 according to Ormston *et al*.,[17] and thyroid-stimulating hormone (TSH) according to Fisher.[18] Furthermore, the reproductive hormone testosterone was measured according to Sigberg.[19]

Collection of Blood Samples

The end of each experiment animals were completely anesthetized by diethyl ether for several minutes, and blood samples were obtained by heart puncture collected in to non-heparinized tubes used in biochemical examination, 4 ml of blood collected from each rat was used to obtain sera (0.5–1.0) ml separated by centrifugation 3000 rpm for 5 min, then they were kept in −20°C until analysis for the measurement of the level of TSH, measurement of the level of T3, measurement of the level of T4, and measurement of the level of testosterone.

RESULTS

Thyroid Hormone Function

Statistical analysis for the effect of ZnO NPs on T3, T4, and TSH serum levels is shown in Figures 1-3. T4 (μg/dl) serum level displayed high significant decrease ($P < 0.01$) in both treated groups (30, 60) mg/kg (3.10 ± 0.01), (2.90 ± 0.02) (μg dl), respectively, at day 7 compared to control groups (3.34 ± 0.01) (μg/dl), at day 14 also showed high significant decrease ($P < 0.01$) at the same concentrations (2.71 ± 0.01), (2.21 ± 0.02) (μg/dl) when compared with control group (3.35 ± 0.01) (μg/dl), in addition to 28 period of time exposing to ZnO NPs observed high significant decrease ($P < 0.01$) of the level of T4 in different concentration (30, 60) mg/kg (1.91 ± 0.02), (1.66 ± 0.01) (μg/dl) compared with control groups (3.36 ± 0.01) (μg/dl), as shown in Figure 1.

Statistical analysis of the level of T3 (ng/ml) showed high significant decrease ($P < 0.01$) in both treated groups (30, 60) mg/kg (0.685 ± 0.007), (0.600 ± 0.010) (ng/ml), respectively, exposed to ZnO NPs for 7 days compared to the control groups (0.786 ± 0.008) (ng/ml), also there was high significant decrease ($P < 0.01$) in the level of T3 at both concentrations (30, 60) mg/kg at 14 days (0.581 ± 0.01), (0.438 ± 0.019) (ng/ml) in comparison to control groups (0.786 ± 0.008), and at 28 days (0.440 ± 0.01), (0.335 ± 0.01) (ng/ml) in comparison to control groups (0.795 ± 0.009) (ng/ml), as shown in Figure 2.

While serum level of TSH (μlU/ml) showed high significant decrease ($P < 0.01$) at different treatment durations exposing to ZnO NPs in both treated groups (30 and 60) mg/kg (0.186 ± 0.007), (0.190 ± 0.010) (ng/ml), respectively, for 7 days compared to the control groups (175 ± 0.008) (ng/ml), also there was high significant decrease ($P < 0.01$) in the level of (TSH) at both concentrations (30 and 60) mg/kg in 14 days (0.188 ± 0.01), (0.195 ± 0.019) (ng/ml), respectively, in comparison to control group (0.175 ± 0.008), and at 28 days (0.191 ± 0.01), (0.198 ± 0.01) (ng ml) in comparison to control group (0.174 ± 0.009) (ng/ml), as shown in Figure 3.

Testosterone

Statistical analysis of the present study of ZnO NPs on testis function is observed in Figure 4 there was a high significant decrease ($P < 0.01$) in testosterone level at 30 and 60 mg/kg for 7 days (160.83 ± 1.16), (149.00 ± 1.29) Ng/dl, respectively, compared to control group (184.67 ± 1.05) Ng/dl. Testosterone level exhibited significant decrease ($P < 0.01$) at day14

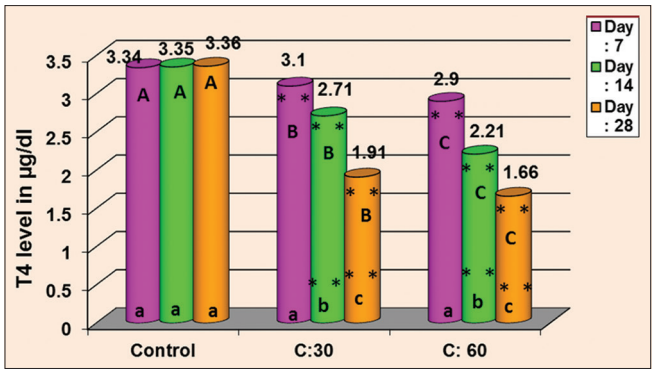

Figure 1: Effect of different concentrations of ZnO NPs (30 and 60) mg/kg on T4 levels of rats with different periods of time (7, 14, and 28) days in comparison with control groups and between treated groups themselves. **High significant decrease (≤0.01). [A,B,C]Represents the significant difference between groups with days as a fixed factor and concentrations as a variable factor. [a,b,c]Represents the significant difference between groups with concentrations as a fixed factor and days as a variable factor

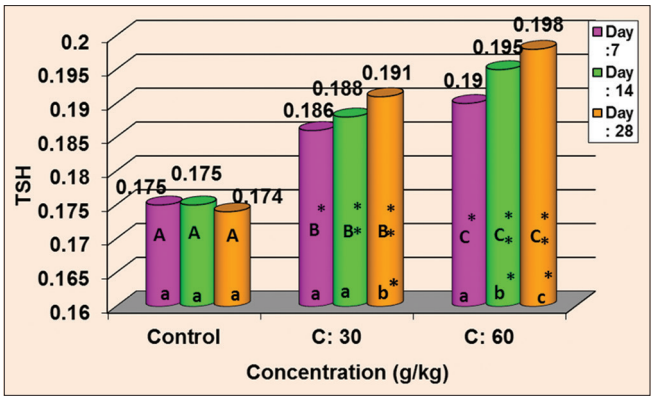

Figure 3: Effect of different concentrations of zinc oxide nanoparticles (30 and 60) mg/kg on thyroid-stimulating hormone levels of rats with different periods of time (7, 14, and 28) days in comparison with control groups and between treated groups themselves. **High significant decrease (≤0.01). [A,B,C]Represents the significant difference between groups with days as a fixed factor and concentrations as a variable factor. [a,b,c]Represents the significant difference between groups with concentrations as a fixed factor and days as a variable factor

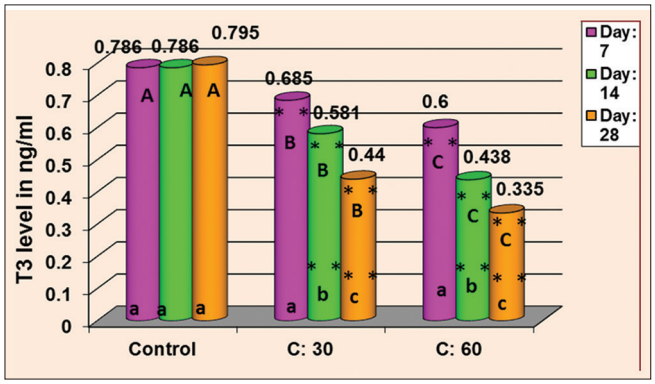

Figure 2: Effect of different concentrations of zinc oxide nanoparticles (30 and 60) mg/kg on T3 levels of rats with different periods of time (7, 14, and 28) days in comparison with control groups and between treated groups themselves. **High significant decrease (≤0.01). [A,B,C]Represents the significant difference between groups with days as a fixed factor and concentrations as a variable factor. [a,b,c]Represents the significant difference between groups with concentrations as a fixed factor and days as a variable factor

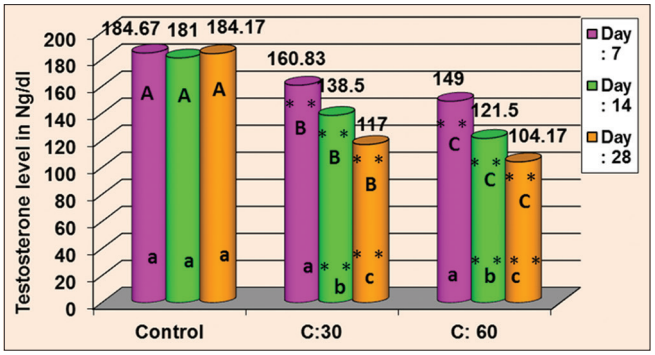

Figure 4: Effect of different concentrations of zinc oxide nanoparticles (30 and 60) mg/kg on testosterone levels of rats with different periods of time (7, 14, and 28) days in comparison with control groups and between treated groups themselves. **High significant decrease (≤0.01). [A,B,C]Represents the significant difference between groups with days as a fixed factor and concentrations as a variable factor. [a,b,c]Represents the significant difference between groups with concentrations as a fixed factor and days as a variable factor

when treated with ZnO NPs (30 and 60) mg/kg (138.50 ± 0.99), (121.50 ± 0.99) Ng/dl when compared with control groups (181.00 ± 1.31) Ng/dl. There was significant decrease ($P < 0.01$) showed in testosterone level at a period of 28 days treatment (30 and 60) mg/kg (117.00 ± 1.52), (104.17 ± 1.42) Ng/dl in comparison to control groups (184.17 ± 1.24) Ng/dl.

DISCUSSION

Results of the present study are in agreement with previous study of ZnO NPs by Cheric and Rafieirad[20] showed that injecting ZnO NPs with three different doses (1.25, 2.5, and 5) mg/kg to 42 male rats divided into 7 groups, the blood samples of experimental with different doses were taken on the 1st, 3rd, 4th, and 15th days after receiving Nano ZnO intraperitoneally (ip) to measure the amount of T3 and T4 hormone levels, results observed that acute injection of Nano ZnO reduced the amount of thyroid hormone T3 and T4, the effect occurred in different doses, but is more significant in the medium and long term, even in small amounts can cause negative effects on the activity of the thyroid gland and disrupt creation of thyroid hormones. Another study by Espanani *et al*.[21] who treated 48 male rats by ZnO NPs ip (5, 10, 20, and 40 mg/kg), after a 21 days period results showed significant increase in TSH hormone level in animals that were treated by high dose of ZnO NP, but those who treated by 5, 10, and 20 mg/kg do not show this effect. Dean *et al*.[22] Found a reduction in thyroid function T3, T4 when he fed chicken with Zn in diet at 73 ppm or 5280 ppm for 1–2 weeks, have demanded that high Zn intake change the production and secretion of thyroid hormones, they suggested that decrease in thyroid hormone function was due to reduced effects of thyroid gland might be induced by the reduced regulatory effect of pituitary gland on thyroid gland, and decreased circulating thyroid hormones

may be indicative of hypothyroidism due to Zinc toxicity. Lu *et al*.[23] Stated that Zn decrease the binding of T3 to its receptor in several preparations *in vitro*.

Kaya *et al*.[24] Conducted the experiment using 130 Hisex Brown laying hens from 56 weeks to 68 weeks of age, then they were divided into five zinc treatment groups (0, 25, 50, 100, and 200) mg/zinc/kg diet respectively, the values of 100 and 200 mg/Zn/kg decreased plasma level of T4 compared to control, while plasma level of T3 was reduced by 100 mg/Zn/kg compared to groups fed less Zn, these data might suggest that a high intake of Zn changes production or secretion of the thyroid hormone.[25] Used 12 lambs and 12 goats that were divided into two equal groups as control and Zn groups in separate experiments, both species of animals in the Zn groups were fed a basal ration supplemented with zinc sulfate adjusted to 250 mg Zn/kg diet showed significant decrease in hormone level T3 and T4.[26] Randomly distributed 120 male rabbits into four groups, the control groups were fed on a basal diet with zinc-free premix, while experimental groups received the basal diet supplemented, Group 1 with 60 mg/kg nano ZnO/kg diet, Group 2 60/kg mg nano ZnO/kg diet, and Group 3 30/kg mg nano ZnO/kg diet, respectively, results observed that rabbits showed no significant changes among the treated groups in respect to serum TSH concentration. In contrast, Baweja *et al*.[27] demonstrated that the levels of TSH were raised after 8 weeks of zinc supplementation in female Wistar rats. Another study that disagree with present report by Shirband *et al*.[28] that administered iron oxide NPs with three different dose (20 μg/kg, 50 μg/kg, and 150 μg/kg) for 15 days to male rats, results showed significant increase in T4 level in groups receiving a dose 50 μg/kg and caused significant decrease in TSH in the group receiving a dose 50 μg/kg and 150 μg/kg. It is likely that NP effects can be applied through the inhibition of endocrine pituitary axis hypothalamus which affects the hypothalamus, and probably due to decreased TSH levels. NPs affect hypothalamic pituitary thyroid axis and therefore affect the level of thyroid hormones that were demonstrated in this study. In a study by[29] used 32 adult male mice treated daily for 35 days: 5, 50, and 300 mg/kg ZnO NPs, respectively, while control group received distilled water orally for 35 consecutive days, results observed significantly changed in 50 and 300 mg/kg ZnO NPs treated mice in epididymal sperm parameters including sperm number, motility, and percentage of abnormality, while significant decrease in seminiferous tubule diameter, seminiferous epithelium height, and maturation arrest was observed at 50 and 300 mg/kg ZnO NPs, this study established that ZNP has cytotoxic actions on testicular germ cells in a dose-dependent manner, NPs might also affect Sertoli cell functions.[30] Who treated 40 female Wistar rats with zirconium oxide NPs with a dose of 100, 200, and 400 ppm injected intraperitoneally showed a significant decrease in level of testosterone at high doses. Reports of a study by Mohammadi *et al*.[31] injected Titanium dioxide TiO NPs intraperitoneally to Wistar rats weighing 150–250 g, 1 ml TiO2 NPs in doses (30 and 50) mg/kg, injection repeated every other day, results showed decrease in testosterone level, could be caused by adverse effects of NPs in the Leydig cells, as Leydig cells are testosterone production factories. Another study by Fuse *et al*.[32] showed that zinc NPs disorder leads to atrophy to the seminiferous tubules and impaired spermatogenesis in male rats. In a study by Shirvani *et al*.[33] was intraperitoneal injected of different doses (25, 50, and 100 mg/kg) of ZnO NPs (25 nm) to male Wistar rats showed decrease but no significant in level of testosterone, synthesis of testosterone is inhibited as a result of responding to the inflammation which is caused by ZnO NPs.[34] Treated rats with ZnO NPs with dose (5, 10, 20, and 40 mg/kg) results revealed significant increase in level of testosterone in blood serum of rats at high dose of ZnO NPs (40 mg/kg), results assured exposing to ZnO NPs damaged to public health and reduced fertility potential. ZnO NPs are related to (ROS), which results in an increase in DNA double-strand breakage and a decrease in sperm motility.[33] Kolesarova *et al*.[35]reported that metal NPs induce changes in reproductive organs, histology of laboratory animals and cause disruption in reproductive cells production and hormones. Thyroid hormone deficiency affects all tissues of the body including multiple endocrine changes that alter growth hormone, corticotrophin, gonadal function, and glucocorticoids, as primary hypothyroidism is associated with hypogonadotropic hypogonadism, so hypothyroidism decrease free testosterone concentration as thyroid hormone affects in sex hormone-binding globulin (SHBG).[36] Alterations in gonadal steroid genesis and pituitary functions have been stated in hypothyroid males, hypothyroidism was found to be associated with an increase in level of total cholesterol and reduction in the levels of testosterone and progesterone without any alteration in the levels of gonadotropins and estradiol, the decline in level of testosterone could be explained by reduction in serum triiodothyronine, a higher rate of alteration of testosterone to estradiol or the further decline in the rate of alteration of progesterone to testosterone.[37] Increasing in TSH level that was caused by ZnO NPs decreased gonadotropin-releasing hormone secretion by negative feedback inhibition that led to LH and FSH reduction, while inhibin hormone that is usually released by Sertoli cells can affect FSH hormone level.[21] Considering the results of the above mentioned studies and the alterations in results from the present study, these outcomes could be related to the dosage of ZnO NPs used, animal species diversity, route of administration of NPs and different durations of exposing to NPs, NPs showed cytotoxic effect on testosterone and thyroid hormone levels in dose and time-dependent manner.

CONCLUSIONS

The results of this study showed that the size, doses, route of administration, and time depended can be a factor that effects thyroid hormone level and testosterone, the decrement in T3 andT4 can be caused by dose and duration of ZnO NPs, decrease in levels of thyroid hormones due to toxic effect of ZnO NPs that effects on the function of thyroid gland or on release of TSH. NPs affects the reproductive system of male by complex and varied mechanisms; results indicate that thyroid hormone deficiency has effects on testosterone level, as hypothyroidism decrease free testosterone concentration, also thyroid hormone has effects on SHBG.

ACKNOWLEDGMENTS

The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq) Baghdad, Iraq, for its support in the present work.

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