Zinc oxide nanoparticles effect on thyroid and testosterone hormones in male rats

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Abstract. Background: Due to their unique properties, zinc oxide nanoparticles (ZnO NPs) gained a broad utilization in nano-based industries and medicine, which may expose human to increased levels of NPs. Zinc oxide (ZnO) is one of the most commonly utilized material in diverse industrial fields such as dyes, additives, rubber, ceramics, chemical fibers, electronics, medical diagnosis, sunscreens, cosmetics, personal care products, and food additives. Nanomaterial describes tiny materials, decreasing the size of the nanoparticles leads to the increase in surface area and provides the absorption possibility for further chemical molecules on the surface and this increases the reactivity of the particles, leads to increase in the effects of toxicity in these materials, ZnO nanoparticles effectively absorb UV-A radiation, as they are used as biomarker for Cancer Treatment and for Biomedical Applications, these nanoparticles display antifungal, and antibacterial effects. Exposure to zinc oxide nanoparticles 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. While Methods : A total of 54 Sprague-Dawley albino adult male rats were divided into nine groups each of 6 rats, daily treated intra-peritoneal with ZnO NPs two different doses (30,60)mg/kg in three different periods of time (7,14 and 28) days, as following : Control groups (group 1,2,3) : respectively received intra-peritoneal injection with distilled water for (7,14, and 28) day, Experimental groups (group 4,5,6) : they were rats respectively received intra-peritoneal dose (60mg/kg) of zinc oxide nanoparticles for (7,14 , and 28) day, and (group 7,8,9) experimental groups: were rats respectively received intra-peritoneal dose (30 mg/kg) of zinc oxide nanoparticles for (7,14, and 28) days. the Result: Data showed high significant decrease(p<0.01) in level of T3 and T4 and level of testosterone also decrease at high and low dose for 7,14 and 28 days,while the level of TSH showed no significant change in all dose and duration of time. Results of the current study suggested the possible time and dose effects disrupting of AgNPs on thyroid gland function and teestes in male rats

Keyword: T3, T4, Testosterone , TSH, ZnO NPs.

1. Introduction

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 three hundred 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
packing [3]. They are also being explored for their potential use as fungicides in agriculture [4]. As well in anticancer drugs and imaging in biomedical applications [5]. Commonly considered to be a material with low toxicity because the zinc oxide 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 nanoparticles [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], Zinc oxide Nanoparticles 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 endrogen 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) reactive oxygen species related with loss of mitochondrial membrane potential , so injection of ZnO NPs produced structural alterations in the seminiferous epithelium and sperm abnormalities in male rats [15].

2. Materials and methods

2.1. Animals

Adult Male Sprague- Dawley albino rats, age about 2.5-3 months, average body weight 200-225 grams . They were obtained from the national center for drug control and research (NCDCR) /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 seven 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 hours light and darkness period, and allowed free access to food 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.

2.2. Preparation of Zinc oxide nanoparticles (ZnO NPs) solution

ZnONPs 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(10-30nm) in diameter. The stock suspension was prepared by dissolving 1gram of powder zinc oxide 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 30mg/kg of ZnO NPs ( low dose ) 60 µl of stock +940 µl of distal water.
2.3. Experimental design

To study the effect of ZnO nanoparticles animals were divided into nine groups with 6 rats each as follows:

Group 1, 2, and 3 (control group); respectively received intraperitoneal injection of distilled water for (7, 14, 28) days. 

Group 4, 5, and 6 (the experimental groups); rats respectively received intraperitoneal dose (60 mg/kg) of zinc oxide nanoparticles for (7, 14, 28) days. 

Group 7, 8, and 9: (the experimental groups); rats respectively received intraperitoneal dose (30 mg/kg) of zinc oxide nanoparticles for (7, 14, 28) days.

2.4. 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 [16], T4 according to [17], and TSH according to [18]. Also, the reproductive hormone testosterone was measured according to [19].

2.5. Collection of Blood Samples:

The end of each experiment animals were completely anaesthetized by diethyl ether for several minutes and blood samples were obtained by heart puncture were 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 -20ºC 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.

2.6. Statistical Analysis

The data obtained were analyzed using two-way analysis of variance (ANOVA) by The Statistical Analysis System-SAS (2012) program followed by LSD test. p<0.01 was considered statistically significant to compare between means in this study.

3. Results

3.1. Thyroid hormone function

Statistical analysis for effect of ZnO NPs on T3, T4, and TSH serum levels showed in figure (1), (2), and (3). T4(µg/dl) displayed high significant decrease(p<0.01) of 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). Showed in figure (1).
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, 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.

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Statistical analysis of T3 showed high significant decrease (p<0.01) on serum level of (T3) (ng/ml) in both treated groups (30,60) mg/kg (0.685±0.007),(0.600±0.010) (ng/ml) respectively exposed to ZnO NPs for 7days 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 showed in figure (2).
(**) 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.

While serum level of TSH (µIU/ml) showed non-significant changes at different treatment durations (7,14,28) days exposing to ZnO NPs at (30,60) mg/kg demonstrated in figure (3).

![Figure 3](image)

**Figure (3):** Effect of different concentrations of ZnO NPs (30 and 60) mg/kg on TSH levels of rats with different periods of time (7, 14, 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.

### 3.2. Testosterone:

Statistical analysis of the present study of ZnO NPs on testis function was showed in figure (4) there was a high significant decrease (p<0.01) in testosterone level at (30, 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 when treated with ZnO NPs (30,60)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 (30,60)mg/kg (117.00±1.52),(104.17±1.42) Ng/dl in comparison to control groups (184.17±1.24) Ng/dl.
Figure (4): Effect of different concentrations of ZnO NPs (30 and 60) mg/kg on testosterone levels of rats with different periods of time (7, 14, 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.

4. Discussion

Results of the present study are in agreement with previous study of ZnO NPs by [20] showed that injecting ZnO NPs with three different doses (1.25, 2.5, 5) mg/kg to 42 male rats divided into 7 groups, the blood samples of experimental with different doses were taken on the first, third, fourth, and fourteenth day after receiving Nano Zinc oxide intraperitoneally (ip) to measure the amount of T3 and T4 hormone levels, results observed that acute injection of NanoZinc oxide 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 [21] whom treated 48 male rats by ZnO NPs intraperitoneally (5, 10, 20, 40 mg/kg), after a 21 day period results showed significant increase in TSH hormone level in animals that were treated by high dose of zinc oxide nanoparticle, but those who treated by 5, 10, 20 mg/kg don’t show this effect.[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. [23] stated that Zn decrease the binding of T3 to its receptor in several preparations in vitro .
[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-1 diet respectively, the values of 100 and 200 mg Zn kg-1 decreased plasma level of T4 compared to control, while plasma level of T3 was reduced by 100 mg Zn kg-1 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 sulphate 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 zinc oxide/kg diet , (group 2)60/kg mg nano zinc oxide/kg diet and (group 3) 30/kg mg nano zinc oxide/kg diet respectively, results observed that rabbits showed no significant changes among the treated groups in respect to serum TSH concentration. In contrast [27] demonstrated that the levels of TSH were raised after 8 weeks of zinc supplementation in female Westar rats. Another study that disagree with present report by [28] that administered iron oxide nanoparticles 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 nanoparticle effects can be applied through the inhibition of endocrine pituitary axis - hypothalamus which affects the hypothalamic, and probably due to decreased TSH levels.

Nanoparticles affect Hypothalamic pituitary thyroid axis and therefore affect the level of thyroid hormones that was demonstrated in this study. The result of this study about Testosterone level deal with previous report [29] used Thirty two adult male mice, 6–8 weeks old, 25–30 g, randomly divided into four groups, experimental groups (1, 2 and 3) received one of the following treatments daily for 35 days: 5, 50 and 300 mg/kg zinc oxide nanoparticles respectively, while control group received distilled water orally for 35 consecutive days, results observed significantly changed in 50 and 300 mg/kg zinc oxide nanoparticles 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 zinc oxide nanoparticles, this study established that ZNP has cytotoxic actions on testicular germ cells in a dose dependent manner, multinucleated giant cell formation and sloughing of immature germ cells from the seminiferous tubules indicates that these NPs might also affect Sertoli cell functions. [30] who treated 40 female wister rats with Zirconium oxide nanoparticles with dose of (100, 200, 400 ppm) injected intra-peritoneally showed significant decrease in level of testosterone at high doses. The result of present study about decrease of testosterone level agree with previous report by [31] were intra-peritoneally injected Titanium dioxide TiO NPs to wistar rats weighing 150-250 g, 1 ml TiO2 NPs in doses (30 and 50) mg/kg, injection repeated every other day, result showed decrease in testosterone level, could be caused by adverse effects of NPs in the Leydig cells, resulting in decreased hormone production (Leydig cells are testosterone production factory), nanoparticles can decrease and disorder secretion in cells by disruptive effect on mitochondria. Another studies by [32] showed that zinc nanoparticles disorder leads to atrophy to the seminiferous tubules and impaired spermatogenesis in male rats. In a study by [33] were intra-peritoneal injected of different doses (25,50,100mg 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 (5,10,20,40 mg/kg) results revealed significant increase in level of testosterone in blood serum of rats at high dose of zinc oxide nanoparticles (40 mg/kg), results assured exposing to ZnO nanoparticles damaged to public health and reduced fertility potential. ZnO NPs are related with (ROS), which results in an increase in DNA double strand breakage and a decrease in sperm motility [33]. [35] Reported that metal NPs induce changes in reproductive organs, histology of laboratory animals and causes 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 affect 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 gonadotrophins 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 zinc nanoparticles, decreased Gonadotrophin Releasing Hormone (GnRH) 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 nanoparticles, NPs showed cytotoxic effect on testosterone and thyroid hormone levels in dose and time dependent manner.

5. Conclusion:

The result of this study showed that the size, doses, rout of administration and time depended can be a factor that effect of thyroid hormone level and testosterone, the decrement in T3 and T4 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 thyroid stimulating hormone (TSH), NPs affect 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 sex hormone binding globulin (SHBG).

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