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Effect of ZnO NPs on Body and Organ Weights in Male Rats

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Abstract

Engineered nanoparticles application in food, such as those used as delivery systems for colors, preservatives flavors, nutrients, and food packaging .Zinc oxide nanoparticles are used in various application include dyes, paints, pigments, medical diagnosis, sunscreens, cosmetics .The present study aims to investigate the side effects of ZnO NPs on the weight of body and organ in male rats .For this study 54 Spargue-Dawley albino adult male rats were classified into three main groups each of 18 rats treated for a particular duration (7,14,28) days respectively. Each group was subdivided into three subgroups each of six rats treated as follows ; group (1) serve as normal control ,group (2,3) intra-peritoneal treated with ZnO NPs (30,60) mg/kg respectively, body weight of all rats was measured before and after the experiment, then rats were dissected at the end of each experiment and the weights of thyroid, liver, testes, and kidneys were measured. Result showed high significant increase ($p<0.01$) in thyroid gland ,body and kidney weights in all different doses (30,60)mg/kg at durations 7,14 and 28 days ,while the weight of liver and testes showed high significant decreament ($p<0.01$) at all doses(30,60)mg/kg for all duration of time(7,14 ,28) days.

Keywords: *ZnO NPs ,thyroid gland, body weight, liver,kidney ,testis weight ,Rats.*

1. Introduction

Nanoparticles are defined as small substances that have at least one dimension in the range of (1–100)nm, the small size and high surface area of Nanoparticles make it principle participant in all features of modern life

application[1]. Zinc oxide and titanium dioxide nanoparticles applied in composition of diversity of products such as auto cleaning glasses, tiles, air and water

filters[2]. Because of extensive application of these particles in several industries, investigation of nanoparticle role in cell growth and survival has more importance, few studies have been done about the effects of the nanoparticles on the male sexual organs and productivity potential, numbers of studies demonstrated that several types of nanoparticles have toxic effects essentially on kidneys, liver and spleen tissues[3]. Thyroid gland is a large endocrine gland that locates in the lower part of neck, it secretes two main thyroid hormone; (T3) and (T4) which are responsible for regulating cell metabolism in human body[4]. The kidney normally plays an important role in the metabolism, degradation and excretion, regulating red blood cell production, blood pressure, blood volume and pH of body fluids[5]. Liver is the main target organ for absorbed materials from gastrointestinal tract before becoming systemic[6]. Testes are two oval organs, a part of the male reproductive system, each testes are

suspended outside the abdomen in a saclike scrotum[5]. Thyroid hormones play in important role in development of male reproductive tract[7]. ZnO NPs induce liver injury, accumulate in the liver of mice after oral administration of (30 nm) of ZnO NPs for 14 days, cause oxidative stress mediated by DNA damage and apoptosis[8]. 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 , injection of ZnO NPs produced structural alterations in the seminiferous epithelium and sperm abnormalities in male rats[9]. NPs can enter the human body through inhalation , ingestion and skin contact or genitourinary tract and become placed in vital organs such as liver, brain and kidneys [10];[11]. Several Studies have shown that nanoparticles may cause alteration or damage cellular processes by passing through cellular membranes to interact with biomolecules leading to damage of DNA and protein[12]. NPs have the ability to induce toxicity has been attributed to their increased surface reactivity [13].The smaller the particles are, the more surface they have per unit mass and the more reactive they are in the cellular environment. It has also been proposed that the size of NP surface area greatly increases their ability to produce reactive oxygen species (ROS)[14].

2. Materials and Methods.

Preparation of Zinc oxide nanoparticles (ZnO NPs) solution

-ZnONPs used in this study was obtained from skyspring nanomaterial's

the ZnONPs characteristic in the following ;

-White to light yellow color powder with 99.8% purity

-particle size (10-30nm)diameter dispersing

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 the following groups :

1. Group of 60 mg/kg of ZnO NPs (high dose) 120 µl of stock +880 µl of distal water
2. Group 30mg/kg of ZnO NPs (low dose) 60 µl of stock +940 µl of distal water [25].

Animal care

54 adult Male Sprague- Dawley albino rats (Rattus norvegicus) ages between 2.5-3 months as mammalian model, the mature male with an average body weight (225-235)gm, animals were purchased from the national center for drug control and research (NCDCR) /ministry of health the animals then transferred to the animal house of the college of science, AL-Mustansyria University , males were kept in clean plastic cages with metal network cover under climate controlled condition of the animal house with 22-25 temperature , 60% humidity, 12 hours light and 12darkness, with free access to food libitum and water.

Experimental design

In this study 54male rats were randomly distributed into nine groups, (7, 14 and 28) days, each group of six rats, they were treated as follows :

Group 1,2and 3 (control group); respectively received intra-peritoneal injection of distilled water for different durations (7,14,28) days .

Group 4, 5, and 6 (the experimental groups) ; the rats respectively received intra-peritoneal dose (60mg/kg) of (ZnO NPs) for different durations (7,14 ,28) days.

Group 7,8 and 9: (the experimental groups); the rats respectively received intra-peritoneal dose (30 mg/kg) of (ZnO NPs) for different durations (7 ,14, 28) days.

Weights Measurements

The end of each experiment was followed by weighing the animals before and after the experiment also weights of thyroid,liver, kidneys and testes were measured for experimental and control groups after rat dissection by diethyl ether for several minutes, the organs washed with normal physiological saline 0.9% (NaCl), blotted with filter paper, weighed and kept in the fixative solution (neutral buffered 10% formalin).

3. Result and Discussion :

3.1 Body weight

The statistical analysis of the present study for ZnO NPs on body weight (gm) in figure (1) showed: increase in the body weight of animal groups exposed to ZnO NPs compared to control groups . 7 days exposing to ZnO NPs demonstrated high significant increase ($p<0.01$) in body weights of experimental treated groups with different concentrations (30 and 60)mg/kg (251.18±0.21), (254.93±0.13)gm respectively when compared with control groups (238.21±0.23)gm.

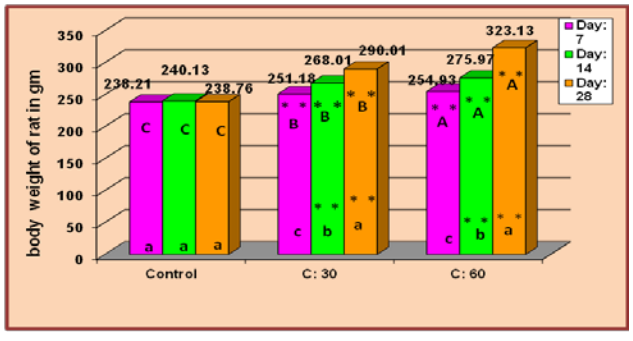


Figure (1) : Effect of different concentrations of ZnO NPs (30 and 60) mg/kg on bodyweights of rats with different periods of time (7, 14, 28) days in comparison with control groups and between treated groups themselves.

(**) high significant increase ($p\leq0.01$) .
(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.

As well, there was high significant increase($p<0.01$)in body weights exposed to ZnO NPs for 14 days of experimental treated groups with concentrations (30 and 60) mg/kg (268.01±0.15), (275.97±0.40) respectively comparing with the control groups (240.13±0.17)gm , also There was high significant increase ($p<0.01$) in body weights exposed to ZnO NPs with concentrations (30 and 60) mg/kg for 28 days(290.01±0.26), (323.31±0.16) gm in comparison with control groups(238.76±0.28)gm.

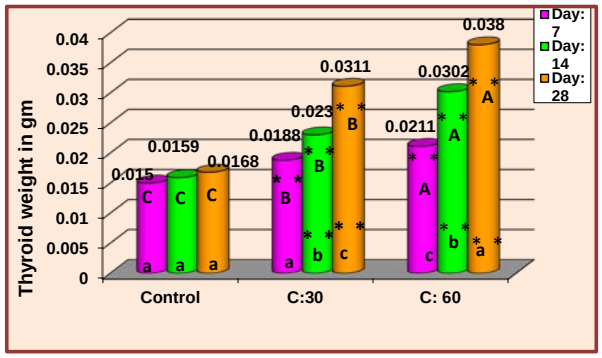
The obtained results of the present study about the increase in body weights agreed with a previous study by[15] were ZnO NPs was added into the basal diet at (0, 50, 500 and 5000) mg/kg, results showed that at(50 and 500) mg/kg nano-ZnOs demonstrated increase in body weight, while at 5000 mg/kg showed decrease in body weight ,it was reported that high dose of ZnO NPs in diet could produce toxicological impact ,but the reduce in body weight at 5000 mg/kg ZnO NPs might partly contribute to the increases in the relative organ weights such as pancreas, brain, and lung, in addition to 5000 mg/kg nano-ZnOs caused damage to hepatic function, altered zinc metabolism in small intestine and led to a significant accumulation of zinc in the liver, pancreas, kidney, and bones. Another study that agreement with the result of present study by[16] were randomly distributed rabbits into four groups. The (control group) was fed on a basal diet with zinc free premix, the other 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, result showed that rabbits in the groups 2 and group 3 had higher body weight, daily weight gain, and daily feed intake, suggesting a better a [17] whom treated rat with different dose of Ag NPs (100,1000 and 5000 mg/kg) for 7, 14 and21 days ,showed significant increase in body weight , which support this study, the increase body weight may refer to possible toxicity of Ag NPs . Also ,Thyroid hormones are supposed to play a role in the regulation of body and fat weight homeostasis by reducing the body fat content, the lack of thyroid hormones is thought to reduce energy expenditure, influence circulating leptin levels indirectly by regulation of adipose tissue mass ,increase the fat mass and possibly increase body weight, so the low level of thyroid hormone increase of fat mass and reduce energy expenditure which lead to weight gain which agree with the present study .bsorption and higher bioavailability of nano-zinc[18]. Another study done by[19] showed decrease in body weight of experimental groups treated with Nano ZnO at concentrations (500 ,1000 ,2000) mg/kg for 14 days. [20] showed significant decrease in body weight of rats at concentration 5 mg/kg ,when treated with ZnO NPs (5,50 and300) mg/kg respectively for 14 days, there reduce in body weight occurred because of the reduce in food consumption after administration and a direct effect of ZnO NPs. [21] administered ZnO-NPs in concentration (10mg/kg) to Wistar rats, results observed non-significant effect on the body weight gain which indicates the lack of toxic signs and mortality in adult rats exposed to ZnO NPs. [22] showed that the weight of body and organ are sensitive indicators of potentially toxic articles in general toxicity studies the suppressed gain body weight affected the total weight of some organs, such as the liver, kidney, and heart, or the relative organ to body weights. [23]whom administered ZnO NPs to pregnant rats by gavage at dose (0, 100, 200, and 400) mg/kg/day, all dams were subjected to a cesarean section on gestational day 20, showed decrease in body weight due to reduction in food consumption at 200,400 mg/kg , toxicity in the dams manifested as significantly decreased body weight after administration of 400 mg/kg/day ZnO NPs . The toxicity of ZnO NPs is associated to their small size, concentration, bio- distribution and high specific surface area [24]. ZnO NPs may lead to an imbalance in lipid metabolism that lead to an increase or decrease in body weight[25].

It is supposed that increase in body weight due to effect of dose and duration of treatment ,the ZnO NPs may accumulate in different animals organs that induce alterations in cellular functions , and consequently alter their metabolic rate.

3.2. Thyroid gland

Result showed that ZnO NPs effects on thyroid weights as it was demonstrated in figure(2), .rats exposed to ZnO NPs for 7 days demonstrated high significant increase($p<0.01$) in thyroid weight of treated groups with concentration (30 and 60) mg/kg (0.0188±0.0002), (0.0211±0.0001)gm respectively compared to control groups(0.015±0.0002)gm, as well, there was high significant increase ($p<0.01$) in thyroid weights of experimental groups treated with ZnO NPs for 14 days in concentration (30 and 60)mg/kg (0.023±0.0002), (0.0302±0.0002)gm respectively when compared to control groups(0.0159 ± 0.0002) gm,



Figure(2): Effect of different concentrations of ZnO NPs (30 and 60) mg/kg on thyroid weight of rats with different periods of time (7, 14, 28) days in comparison with control groups and between treated groups themselves.

(**) high significant increase (≤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.

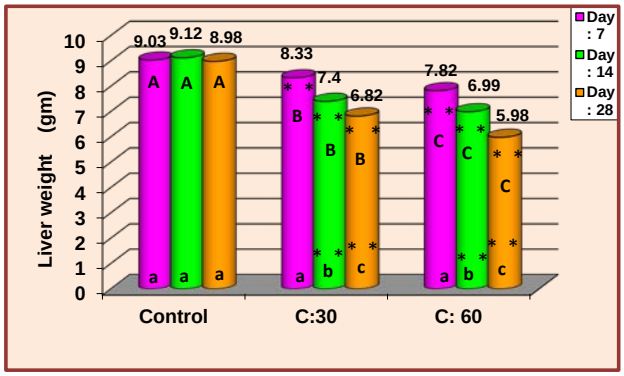
also at 28 days period of time there was high significant increase($p<0.01$) in thyroid weights (0.0311±0.0001),(0.038±0.0001)gm respectively compared with control groups (0.0168±0.0003)gm.

Results of the present study about the elevation in thyroid weight is in agreement with a previous study by[26] who reported significant increase in thyroid weight in response to hypothyroidism and hypertrophy in thyroid gland. [27] who showed that thyroid weight in hypothyroidism rats was significant enlarged. Another study by[28] showed that animals were orally exposed to thyroid disrupting compounds like ,ammonium perchlorate with different doses of (0.3, 3.0, and 30.0 mg/kg/day) ,shown a significant thyroid hypertrophy and increase in thyroid gland weight .actually the weight ,size and histology of thyroid gland affected by production of thyroxin and its functional status .,where some disorder of thyroid gland such as overactive or underactive thyroid gland are established by enlargement of thyroid gland as a part of compensatory mechanism to maintain of thyroid hormone homeostasis [29].

Results observe hat dose and time depended of ZnO NPs increase the weight of thyroid with the increment of time and doses of NPs.

3.3. The Liver

The statistical analysis of present study showed high significant decrease($p<0.01$) in liver weights of treated groups with ZnO NPs at both concentration (30 and 60) mg/kg (8.33±0.03),(7.82±0.02) with a period of 7days in comparison with the control groups(9.03 ± 0.04) ,the liver weight of experimental groups at 14 days treated with ZnO NPs (30 ,60) mg/kg showed high significant decrease($p<0.01$). (7.40±0.02),(6.99±0.02)gm in comparison to control groups (9.12±0.02),



Figure(3): Effect of different concentrations of ZnO NPs (30 and 60) mg/kg on liver weight 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.

also at 28 days of treatment demonstrated high significant decrease(<0.01) in weights of liver in (30 and 60)mg/kg (6.82±0.02),(5.98±0.03)gm compared with control groups (8.98±0.02)gm which is shown in figure(3) .

The present study about decrement of liver weight deal with preceding report by [23] pregnant rats were administered ZnO NPs by gavage at different doses (0, 100, 200, and 400) mg/kg/day, that lead to decrease in liver weights after administration of 400 mg/kg/day NPs. [30] showed that inhalation exposure to ferric oxide (Fe2O3 NPs) and (ZnO NPs) in Male Wistar rats, were consecutively treated with Fe2O3 at (8.5 mg/kg), and ZnO NPs at(2.5 mg/kg), two times daily for 3 days, Fe2O3-treated group, iron (Fe) content in liver and lung tissues was significantly increased at 36 h , the ZnO-treated group, zinc (Zn) content in liver tissues was significantly increased at 12 h and further increased at 36 h it was reported that ZnO NPs damaged the organs such as lung, liver and pancreas ,Histo-pathological showed that NPs caused severe damage in liver. [31] were added ZnO NPs in feed at (300–600 mg/kg) , showed that the liver and kidney injury occurred when orally administered of ZnO NPs to Sprague Dawley rats . Another previous study identified the liver as the main target organ of ZnO NPs [32]. Because the liver metabolizes drugs via its constitutive cytochrome P450 (CYP450) enzyme system, we speculated that a clinical dose of ZnO NPs might produce adverse effects, as Zn accumulates in the liver and decreases the elimination rate of other drugs, leading to their accumulation and associated toxic side effects[33].

[19] Observed that the inhibited body weight gain affected the total weight of some organs, such as the liver. A preceding study by[21] whom administered of ZnO-NPs suspended in distilled water to Wistar rats at dose of 10 mg/ kg through oral gavage for 5 repeated days results showed no- significant change in liver weight, administration of ZnO NPs orally induced a non-significant rise of zinc concentration in the liver and kidney. Although the number of published oral exposure studies has increased during the recent years, only very few well performed studies on intestinal absorption are available these result disagree with result of present study.[15] were adding of ZnO NPs to basal diet at (0,50,500,500 mg/kg) in mice, demonstrated that long term exposure to 50 and 500 mg/kg nano-ZnO diets showed minimal toxicity. However, high dose of nano-ZnOs (5000 mg/kg) caused toxicity on development and reduction in liver weight but no significant, due to toxicity of ZnO NPs ,damaged hepatic function and altered zinc metabolism in small intestine and led to a significant accumulation of zinc in the liver which agree with present study ,damaged hepatic function and altered zinc metabolism in small intestine and led to a significant accumulation of zinc in the liver, pancreas, kidney, and bones.

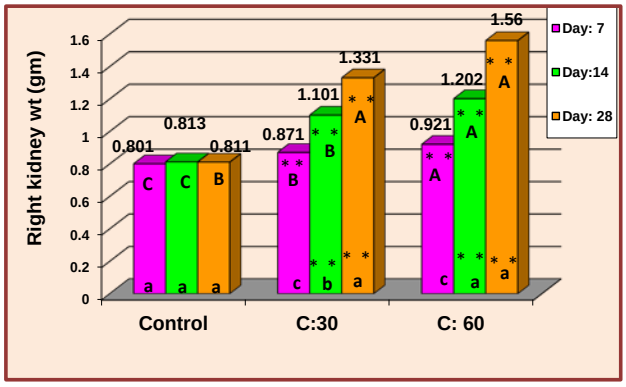
[20] used 32 adult male Wistar rats received 5, 50 and 300 mg/kg ZnO NPs respectively for 14 days , result observed increment in liver weight at doses (5 and 50 mg/kg) of ZnO NPs which disagree with present study, the increase in liver weight is due to accumulation of ZnO NPs in liver or because accumulation of fat RBC and leukocyte can increase liver weight, the vacuolization of hepatocytes (fat deposits), destruction of lobular structure and infiltration of leukocytes indicate necrosis which is effected by ZnO NPs. [34] Showed that ZnO NPs can interact with proteins and enzymes in the interstitial tissue of the liver,

interfering with the antioxidant defense mechanism and leading to generation of (ROS), which may induce an inflammatory response . [35] Showed that ZnO NPs accumulate significantly in the livers of mice after oral administration of 300 nm ZnO NPs for 14 days ,causes pathological lesions in the liver, resulting in oxidative stress mediated by DNA damage and liver cell apoptosis , ZnO NPs were found induce oxidative stress indicated by an increase in lipid peroxidation. in addition to apoptosis, necrosis also observed in liver tissues of ZnO NPs treated animals. [36] found that ZnO NPs were the most cytotoxic to A549 cells, as assessed by DNA fragmentation and apoptosis experiments .both necrosis and apoptosis has been stated to occur in cells treated with ZnO NPs[37] investigated that ZnO NPs induced both necrosis and apoptosis in cells. many study reported that the toxicity of NPs depends on the oxidative stress damage to DNA .However, other mechanisms must also be considered, for example the damaged liver cells, such enzymes that found inside the hepatocytes are released into the blood so, a high amount of these enzymes indicates damage of liver cells.

The results about decrement in liver weight indicating a time and dose dependent of ZnO NPs effect on liver weight that causes damage to liver cells and release of liver enzyme in to blood stream .

3.4. The Kidney

The statistical analysis of the weight of right kidney is shown in figure (4) the rats injected with ZnO NPs intra-peritoneal with both doses(30 ,60 mg/kg) demonstrated high significant increase ($p<0.01$) in weight of right kidney (0.871±0.002)(0.921±0.001)gm respectively at day 7 when compared with the control group (0.801±0.0001) gm, at 14 period of time results showed high significant increase ($p<0.01$) in weight of right kidney (1.101±0.003), (1.202±0.002)gm respectively when compared with the control group(0.813±0.0001)gm,and at 28 period of time observed high significant increase ($p<0.01$) in weight of right kidney (1.331±0.002),(1.56±0.001)gm when compared with the control group(0.811±0.0001)gm. Statistical analysis of the weight of left kidney is shown in figure (5) the rats injected with ZnO NPs intra-peritoneal with both doses(30 ,60 mg/kg) demonstrated high significant increase ($p<0.01$) in weight of left kidney at 7 days(0.861±0.002),(0.941±0.001)gm respectively when compared with the control group(0.812±0.0001)gm, also at 14 days period of time showed high significant increase ($p<0.01$) in weight of left kidney (1.162±0.002),(1.212 ±0.002) gm respectively when compared with the control group(0.812±0.0001)gm, in addition to day 28 of treatment showed high significant increase ($p<0.01$) in weight of left kidney ,(1.310±0.001),(1.581±0.002) respectively gm compared with control groups (0.822±0.001) gm .

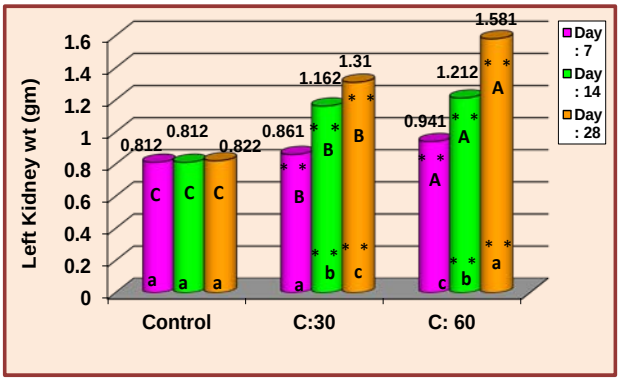


Figure(4): Effect of different concentrations of ZnO NPs (30 and 60) mg/kg on right kidney weight of rats with different periods of time (7, 14, 28) days in comparison with control groups and between treated groups themselves.

(**) high significant increase (≤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(5): Effect of different concentrations of ZnO NPs (30 and 60) mg/kg on left kidney weight of rats with different periods of time (7, 14, 28) days in comparison with control groups and between treated groups themselves. (**) high significant increase (≤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.

Nanoparticles have been shown to enter systemic circulation then, they have the possible to cause organ damage throughout the body. Those organs with extensive blood supply, such as liver, kidneys , and spleen are especially susceptible. The kidneys play a mainly important role as they are capable of filtering NPs out of the systemic circulation. so, they are increasingly exposed to damage through those NPs they have filtered from blood.

the previous study about kidney weight by[38] showed were intra-peritoneal injection of ZnO NPs with different dose (50, 100, 200, 300) mg/kg to mice after (8, 15 and 30) days post injection showed kidney change including degeneration of proximal and distal tubules and accumulation of inflammation cells (neutrophils and eosinophils) in glomerular capillaries. [15] were nano-ZnOs added into the basal diet at 0, 50, 500 and 5000 mg/kg. Results indicated that added 50 and 500 mg/kg nano-ZnOs showed minimal toxicity. But at 5000 mg/kg nano-ZnOs significantly increase but non-significant in kidney weight of mice. Due to ZnO NPs caused toxicity on development, and altered the zinc metabolism and bio-distribution in mice. And investigate the toxicity of ZnO NPs, which can simply enter cells and lead to oxidative stress.

A study by [39] were oral administration of ZnO NPs at (333.33mg/kg) for five days to mice showed ZnO NPs can cause hydropic degeneration in epithelial cells, necrosis and swelling of epithelial cells of proximal tubules in the kidney tissues of mice treated with ZnO NPs which demonstrated that ZnO NPs toxicological effects on kidney and cause serous inflammation.

ZnO NPs induce oxidative stress which cause kidney damage, oxidative stress and lipid peroxidation play important role in toxicity of ZnO NPs[40]. according to radioactive ZnO experiments, NPs showed retention in the lung, followed by retention in the kidney and liver after intravenous administration [41].

In order to state any comment about nephrotoxicity, seeking of the above factors and also renal tissue structure, because kidney is one of the organs to collect and disposal of waste materials , ZnO nanoparticles have greater effects on Kidney function.

the variations between results of present study and previous study about Kidney weight may be depends on the size of NPs, rote of administration ,concentration and duration of the doses .

The kidneys receive blood from the renal arteries, which branch directly from the dorsal aorta. Despite their relatively small size, the kidneys receive about 20% of the entire cardiac output, making the organs highly susceptible to xenobiotics such as NPs[42]. In theory, both the glomerular structures (during plasma filtration) and tubular epithelial cells may be exposed to NPs. Since the major function of the kidneys is to eliminate a variety of potentially harmful substances (including the potential excretion of NPs , these organs are extremely important targets for investigation with regard to nanoparticle exposure and hazard [43].

3.5. The Testes

The statistical analysis of present study about the weight of right and left testis showed in figure (3-25) , (3-26) respectively, right testis weight displayed high significant decrease($p<0.01$)at 7 days treated with 30 ,60 mg/kg (1.441±0.001) ,(1.392±0.002) gm compare with control groups(1.513±0.002)gm, as well as there was high significant decrease in weight of left testis at 7 days at (30,60) mg/kg (1.452±0.002), (1.381±0.002) when compared with control groups (1.523±0.002) at 14 and 28 days the weight of right testis showed high significant decrease in weight ($p<0.01$) when treated with ZnO NPs

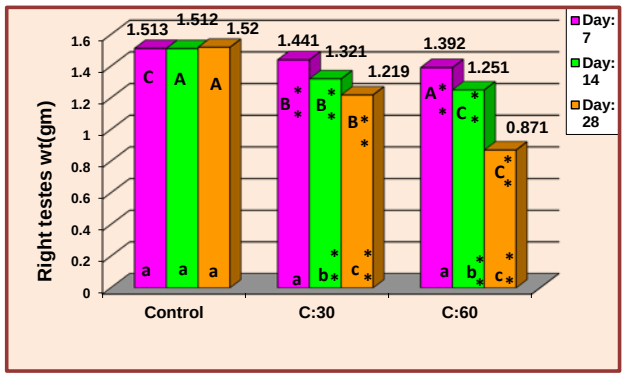
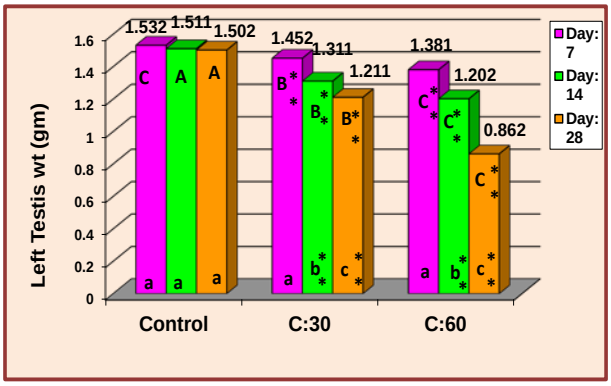


Figure (5): Effect of different concentrations of ZnO NPs (30 and 60) mg/kg on right testis weights of rats with different periods of time (7, 14, 28) days in comparison with control groups and between treated groups themselves.

(**) 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.

with concentration (30,60) mg/kg (1.321±0.002), (1.251±0.001)gm at day 14 and (1.219±0.002), (0.871±0.001)gm at day 28 in comparison with control groups (1.512±0.002),(1.520±0.001)gm respectively . The weight of left testis at 14 and 28 days showed high significant decrease when treated with ZnO NPs at (30,60) mg/kg (1.311±0.001) , (1.202±0.002) gm respectively compared to control groups (1.511±0.002)gm at day 14,while results were (1.211±0.001) ,(0.862±0.002) gm respectively compared to control groups (1.511±0.002) gm at day 28.



Figure(3-26): Effect of different concentrations of ZnO NPs (30 and 60) mg/kg on left testis weights of rats with different periods of time (7, 14, 28) days in comparison with control groups and between treated groups themselves.

(**) high significant increase (≤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.

The present study about reduce of weight in right and left testis agree with another study by [44] whom treated mice with ZnO NPs daily for 35 days at (5,50,300) mg/kg showed the weight of testicular weight at dose 5,50 was slightly less than control groups were at dose 300 showed a significant decrease in testicular weight, ZnO NPs also caused a significant decrease in seminiferous tubule diameter, seminiferous epithelium height and maturation . [45] were mice divided into five groups which intra-peritoneal received of ZnO NPs with different dose (250,500,700 mg/kg/day) , The results shown a significant changes in cell types of testis tissue that were treated with ZnO NPs nanoparticle, These changes such as reduction and loss of cells in seminiferous tubules in testicular tissue, spermatogonia in mice treated with ZnOn (500 and 700 mg/kg/day doses) were significantly decreased. Many study that indicated ZnO NPs induce testicular damage and cytotoxic effects on testicular germ cells in a dose dependent manner in mice. [46] mice were treated intravenously with TiO2 NPs (0, 2, ,10 mg/kg) once per week for four weeks followed by sacrificing nine days after the last injection, TiO2 NPs significantly reduced testis weight These results indicate that TiO2 NPs induce hazardous effect on testicular system both quantity of sperm head numbers and quality of sperm cells which contract with present study. [15] were treated mice with ZnO NPs added to basal diet at different doses (0, 50, 500 and 5000) mg/kg. result showed not changed the relative weight of testis that disagree with present study. Level of (Zn) are very high in the male reproductive system and seminal fluid[47]. [48] found that NPs can affect leydig cells mitochondrial activity and thus lead to reduce their secretory activity . the NPs increase the releasing of free oxygen and this enhance the oxidation of macromolecules such as proteins and finally leading to reduce the numbers of leydig cells and decrease the production of testosterone. A previous study reported that metal NPs induce changes in reproductive organs, histology of laboratory animals and in causing disruption in reproductive cells production and hormones[49]. ZnO NPs are related with (ROS), which results in an increase in DNA double strand breakage and a decrease in sperm motility, there was several hypotheses that suggest ROS may penetrate across the cell membrane and inhibit the activity of some vital enzymes such as , glucose 6-phosphate dehydrogenase G6PDH, the ROS may lead to mutations such as point mutations and polymorphism and thus lead to decrease semen quality, the results of morphometric measurements show that nanoparticles decrease the diameter of germinal epithelium and the size of tubules and therefore cause apoptosis [25].

The present study showed that the NPs inhibit endocrine system function by blocking of pituitary hypothalamus axis and this may decrease level of GnRH leading into reduction in Testosterone level causing effects on spermatogenesis.

4. Conclusions

Nanoparticles may display either acute or chronic toxicity, but the latter type is the most important in foods since relatively low levels of nanoparticles are likely to be consumed over an extended period. In general, the toxicity of ingested nanoparticles depends on their ability to damage cells or organs within humans, The administration of ZnO NPs in rats induce major changes in body and organ weight and feed intake. perhaps does of ZnO NPs

cause many toxicity and certain disturbances in the body. However more researches are necessary on the effect of these nanoparticles on the organs. Moreover additional studies should be treated in different periods and number of injections by examining oxidative stress. our data showed that exposure of male rats to ZnO NPs elicited oxidative stress response in the kidneys, Liver and other organ .

References

[1] M. Fartkhooni, F., Noori A., Momayez M., Sadeghi L., Shirani K., and Y. Babadi, V, "The effects of nano titanium dioxide (TiO2) in spermatogenesis in wistar rat", European Journal of Experimental Biology, 3(4), 2013, 145-149.

[2] Kale, R. D., and Meena, C. R. "Synthesis of Titanium dioxide Nanoparticles and Application on Nylon fabric using Layer by Layer technique for Antimicrobial Property", Advances in Applied Science Research, 3(5), 2012, 3073-3080.

[3] Wang, B., Feng, W., Wang, M., Wang, T., Gu, Y., Zhu, M., Ouyang, H., Shi, J., Zhang, F., Zhao, Y. and Chai, Z. "Acute toxicological impact of nano- and submicro-scaled zinc oxide powder on healthy adult mice", Journal of Nanoparticle Research, 10(2), 2008, 263-276.

[4] Apriletti, J.W., Ribeiro, R.C., Wagner, R.L., Feng, W., Webb, P., Kushner, P.J., West, B.L., Nilsson, S., Scanlan, T.S., Fletterick, R.J. and Baxter, J.D. "Molecular and structural biology of thyroid hormone receptors", Clinical and Experimental Pharmacology and Physiology, 25(S1), 1998 , 2-11.

[5] Shier, D., Butter, J. and Lewis, R. Hole's Essentials of Human Anatomy and Physiology, 9th edition, McGraw Hill Company, New York, 2006.

[6] Barrett, K.E., Boitano, S., Barman, S.M. and Brooks, H.L. Ganong's Review of Medical Physiology. 24th ed, McGraw Hill Company, Inc., New York, 2012.

[7] Choksi, N. Y., Jahnke, G. D., St. Hilaire, C., and Shelby, M. "Role of thyroid hormones in human and laboratory animal reproductive health", Birth Defects Research Part B: Developmental and Reproductive Toxicology, 68(6), 2003, 479-491.

[8] Srivastav, A.K., Kumar, M., Ansari, N.G., Jain, A.K., Shankar, J., Arjaria, N., Jagdale, P. and Singh, D. "A comprehensive toxicity study of zinc oxide nanoparticles versus their bulk in Wistar rats: Toxicity study of zinc oxide nanoparticles". Human & experimental toxicology, 35(12), 2016, 1286-1304.

[9] Han, Z., Yan, Q., Ge, W., Liu, Z.G., Gurunathan, S., De Felici, M., Shen, W. and Zhang, X.F." Cytotoxic effects of ZnO nanoparticles on mouse testicular cells", International journal of nanomedicine, 11, 2016, p 5187.

[10] Chithrani, B. D., Stewart, J., Allen, C., and Jaffray, D. A. "Intracellular uptake, transport, and processing of nanostructures in cancer cells", Nanomedicine: Nanotechnology, Biology and Medicine, 5(2), 2009 ,118-127.

[11] Li, S. Q., Zhu, R. R., Zhu, H., Xue, M., Sun, X. Y., Yao, S. D., and Wang, S. L. "Nanotoxicity of TiO2 nanoparticles to erythrocyte in vitro", Food and chemical toxicology, 46(12), 2008, 3626-3631.

[12] Ahamed, M., Karns, M., Goodson, M., Rowe, J., Hussain, S. M., Schlager, J. J., and Hong, Y. "DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells", Toxicology and applied pharmacology, 233(3), 2008 ,404-410.

[13] Colvin, V. L. "The potential environmental impact of engineered nanomaterials", Nature biotechnology, 21(10), 2003, 1166.

[14] Møller, P., Jacobsen, N.R., Folkmann, J.K., Danielsen, P.H., Mikkelsen, L., Hemmingsen, J.G., Vesterdal, L.K., Forchhammer, L., Wallin, H. and Loft, S.,. "Role of oxidative damage in toxicity of particulates". Free radical research, 44(1), 2010, 1-46.

[15] Wang, C., Lu, J., Zhou, L., Li, J., Xu, J., Li, W., Zhang, L., Zhong, X. and Wang, T. "Effects of long-

term exposure to zinc oxide nanoparticles on development, zinc metabolism and biodistribution of minerals (Zn, Fe, Cu, Mn) in mice", PloS one, 11(10), 2016, 0164434.

[16] Hassan, F. A., Mahmoud, R., and El-Araby, I. E. "Growth Performance, Serum Biochemical, Economic Evaluation and IL6 Gene Expression in Growing Rabbits Fed Diets Supplemented with Zinc Nanoparticles", Zagazig Veterinary Journal (Zag. Vet. J.), 45(3), 2017.

[17]Adeyemi, O. S., and Adewumi, I. "Biochemical evaluation of silver nanoparticles in Wistar rats", International scholarly research notices, 2014.

[18] Syed , M. A., Thompson, M. P., Pachucki, J., and Burmeister, L. A. "The effect of thyroid hormone on size of fat depots accounts for most of the changes in leptin mRNA and serum levels in the rat", Thyroid, 9(5), 1999, 503-512.

[19] Ko, J.W., Hong, E.T., Lee, I.C., Park, S.H., Park, J.I., Seong, N.W., Hong, J.S., Yun, H.I. and Kim, J.C. "Evaluation of 2-week repeated oral dose toxicity of 100 nm zinc oxide nanoparticles in rats", Laboratory animal research, 31(3), 2015, 139-147.

[20] Mansouri, E., Khorsandi, L., Orazizadeh, M., and Jozi, Z. "Dose-dependent hepatotoxicity effects of Zinc oxide nanoparticles", Nanomedicine Journal, 2(4), 2015, 273-282.

[21] Ben-Slama, I., Mrad, I., Rihane, N., Mir, L. E., and Sakly, M. "Sub-Acute Oral Toxicity of Zinc Oxide Nanoparticles in Male Rats", J Nanomed Nanotechnol 6: 284, 2015, doi: 10.4172/2157-7439.

[22] Andersen, H., Larsen, S., Spliid, H., and Christensen, N. D. "Multivariate statistical analysis of organ weights in toxicity studies",Toxicology, 136(2-3), 1999, 67-77.

[23] Hong, J.S., Park, M.K., Kim, M.S., Lim, J.H., Park, G.J., Maeng, E.H., Shin, J.H., Kim, M.K., Jeong, J., Park, J.A. and Kim, J.C."Prenatal development toxicity study of zinc oxide nanoparticles in rats", International journal of nanomedicine, 9(2), 2014, 159.

[24] Valdiglesias, V., Costa, C., Kiliç, G., Costa, S., Pásaro, E., Laffon, B., and Teixeira, J. P. "Neuronal cytotoxicity and genotoxicity induced by zinc oxide nanoparticles", Environment international, 55, 2013, 92-100.

[25] Shirvani, H., Noori, A., and Mashayekh, A. M. "The Effect of ZnO Nanoparticles on the Growth and Puberty of Newborn Male Wistar Rats", International Journal of Basic Sciences & Applied Research, 3, 2014, 180-185.

[26] Soukup, T., Zacharova, G., Smerdu, V., and Jirmanova, I. "Body, heart, thyroid gland and skeletal muscle weight changes in rats with altered thyroid status". Physiological research, 50(6), 2001, 619-626.

[27] Ibrahim, H. S., Rabeh, N. M., and ELden, A. A. S. "Effect of Selenium and Zinc Supplementation on Hypothyroidism in Rats", (2016).

[28] Christian, M. S., and Trenton, N. A. "Evaluation of thyroid function in neonatal and adult rats: The neglected endocrine mode of action", Pure and applied chemistry, 75(11-12), 2003 , 2055-2068.

[29] Chaudhry, Q., and Castle, L. "Food applications of nanotechnologies: an overview of opportunities and challenges for developing countries",Trends in Food Science & Technology, 22(11), 2011, 595-603.

[30] Wan, L., Wang, L., Ding, W., and Zhang, F. "Acute toxicity of ferric oxide and zinc oxide nanoparticles in rats", Journal of nanoscience and nanotechnology, 10(12), 2010, 8617-8624.

[31] Tang, H. Q., Xu, M., Rong, Q., Jin, R. W., Liu, Q. J., and Li, Y. L. "The effect of ZnO nanoparticles on liver function in rats",International journal of nanomedicine, 11, 2016, 4275.

[32] Liu-Sheng, H., Xiao-Shan, Y., and De-Chang, W. "Age-dependent variation of zinc-65 metabolism in LACA mice", International journal of radiation biology, 60(6), 1991, 907-916.

[33] Nebert, D. W., and Russell, D. W. "Clinical importance of the cytochromes P450",The Lancet, 360(9340), 2002, 1155-1162.

[34] Johar, D., Roth, J.C., Bay, G.H., Walker, J.N., Kroczak, T.J., Los, M., "Inflammatory response, reactive oxygen species, programmed (necrotic-like and apoptotic) cell death and cancer", Rocz. Akad. Med. Bialymst, 49, 2004, 31–39.

[35] Sharma, V., Singh, P., Pandey, A. K., and Dhawan, A. "Induction of oxidative stress, DNA damage and apoptosis in mouse liver after sub-acute oral exposure to zinc oxide nanoparticles" Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 745(1), 2012, 84-91.

[36] Park, S., Lee, Y.K., Jung, M., Kim, K.H., Chung, N., Ahn, E.K., Lim, Y. and Lee, K.H. "Cellular toxicity of various inhalable metal nanoparticles on human alveolar epithelial cells", Inhalation toxicology, 19(1), 2007 ,59-65.

[37] Wilhelmi, V., Fischer, U., Weighardt, H., Schulze-Osthoff, K., Nickel, C., Stahlmecke, B., Kuhlbusch, T.A., Scherbart, A.M., Esser, C., Schins, R.P. and Albrecht, C. "Zinc oxide nanoparticles induce necrosis and apoptosis in macrophages in a p47phox-and Nrf2-independent manner", PloS one, 8(6), 2013 ,65704.

[38] Noori, A., and Karimi, F. "Effects of zinc oxide nanoparticles on renal function in mice",KAUMS Journal (FEYZ), 16(7), 2013, 603-604.

[39] Esmaeillou, M., Moharamnejad, M., Hsankhani, R., Tehrani, A. A., and Maadi, H. "Toxicity of ZnO nanoparticles in healthy adult mice", Environmental toxicology and pharmacology, 35(1), 2013,67-71.

[40] Guan, R., Kang, T., Lu, F., Zhang, Z., Shen, H., andLiu, M. "Cytotoxicity, oxidative stress, and genotoxicity in human hepatocyte and embryonic kidney cells exposed to ZnO nanoparticles", Nanoscale research letters, 7(1), 2012, 602.

[41] Chen, J.K., Shih, M.H., Peir, J.J., Liu, C.H., Chou, F.I., Lai, W.H., Chang, L.W., Lin, P., Wang, M.Y., Yang, M.H. and Yang, C.S. "The use of radioactive zinc oxide nanoparticles in determination of their tissue concentrations following intravenous administration in mice", Analyst, 135(7), 2010, 1742-1746.

[42] L'Azou, B., Jorly, J., On, D., Sellier, E., Moisan, F., Fleury-Feith, J., Cambar, J., Brochard, P., Ohayon-Courtès, C. "In vitro effects of nanoparticles on renal cells", Part. Fibre Toxicol. 5, 2008, 651–663.

[43] Kermanizadeh, A., Vranic, S., Boland, S., Moreau, K., Baeza-Squiban, A., Gaiser, B. K., and Stone, V. "An in vitro assessment of panel of engineered nanomaterials using a human renal cell line: cytotoxicity, pro-inflammatory response, oxidative stress and genotoxicity", BMC nephrology, 14(1), 2013, 96.

[44] Talebi, A. R., Khorsandi, L., and Moridian, M. "The effect of zinc oxide nanoparticles on mouse spermatogenesis", Journal of assisted reproduction and genetics, 30(9), 2013, 1203-1209.

[45] Mozaffari, Z., Parivar, K., Roodbari, N. H., and Irani, S. "Histopathological evaluation of the toxic effects of zinc oxide (ZnO) nanoparticles on testicular tissue of NMRI adult mice", Adv Stud Biol, 7, 2015, 275-291.

[46] Miura, N., Ohtani, K., Hasegawa, T., Hojo, R., Yanagiba, Y., Suzuki, T., Suda, M. and Wang, R.S. "Hazardous effects of titanium dioxide nanoparticles on testicular function in mice", Fundamental Toxicological Sciences, 1(3), 2014, 81-85.

[47] Ebisch, I. M. W., Thomas, C. M. G., Peters, W. H. M., Braat, D. D. M., and Steegers-Theunissen, R. P. M. "The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility", Human reproduction update, 13(2), 2006, 163-174.

[48] Carlson, C., Hussain, S. M., Schrand, A. M., K. Braydich-Stolle, L., Hess, K. L., Jones, R. L., and Schlager, J. J. "Unique cellular interaction of silver

nanoparticles: size-dependent generation of reactive oxygen species", The journal of physical chemistry B, 112(43), 2008, 13608-13619.

[49] Kolesarova, A., Capcarova, M., Sirotkin, A., Medvedova, M., and Kovacik, J. "Cobalt-induced changes in the IGF-I and progesterone release, expression of proliferation-and apoptosis-related peptides in porcine ovarian granulosa cells in vitro", Journal of Environmental Science and Health Part A, 45(7), 2010, 810-817.