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The effects of nano titanium dioxide (TiO_2) in spermatogenesis in wistar rat

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ABSTRACT

Nanoparticles have widespread application in all aspects of modern life because of unique features of their as small size and high surface area. Their features especially high surface area cause it is very reactive and toxic. They can damage human and animal cells by increasing oxidative stress mechanism. Nanotitanium dioxide (TiO_2) having different capabilities such as robust oxidation, biocompatibility, photocatalytic properties frequently used in a wide range of sciences, including pharmaceuticals, cosmetics, medicine and engineering. Wide application of this materials resulted wide exposing of human and animals to this, so analysis of its toxicity and distribution in the body is important. This study investigates the effects of TiO_2 nanoparticle on the concentration of sexual hormones such as LH, FSH and testosterone. Results showed significant alteration in LH and Testosterone concentration, but histological studies doesn't show any important changes in testis tissue, count of Spermatogonia, spermatocytes and spermatid that is interestingly different from other study.

Key words: Titanium dioxide nanoparticle, fertility potential, testosterone, LH hormone, Testosterone.

INTRODUCTION

Nanoparticles based on physical and chemical properties and special shape, size and surface area to volume ratio are unique for biological, medical and industrial applications. One of the most useful features of this is high surface area of nanoparticles that cause its widespread application in medical science and production of nano based drugs as a cure for some of the Incurable disease such as cancer (Jani et al. 1990, Garg et al. 2011).

But published literatures showed material at the nano size has relatively greater toxicity rather than large sizes materials, because nanoparticles are highly reactive and cause oxidative stress in human and animals. Previous researches confirmed exposing to nanoparticles crate malignant brain damages in fishes (Davis 2006, Fazilati 2013). Nanoparticles can pass through cell membrane easily and even pass through blood-brain barrier and blood-testes barrier (McAuliffe et al. 2007, Dhamia et al. 2013), so it can affect all organs of the body (Yousefi Babadi et al.

2012). Nanoparticles can enter the bloodstream and reach to the organs (including the brain, heart, kidneys) rapidly by blood circulation (Yousefi Babadi et al. 2012).

Result showed the zinc oxide and titanium dioxide nanoparticles cause the production of free radicals in skin cells and can lead to damage in DNA and can alter protein structure that may be it is important reason for cancer and tumors (Praetorius et al. 2007, Ghorbanzadeh et al. 2012).

Nowadays zinc oxide and titanium dioxide nanoparticles applied in composition of variety of products such as auto cleaning glasses, tiles and air and water filters (Kale et al. 2012). Because of widespread application of these particlesin various industries, investigation of nanoparticle role in cell growth and survival has more importance. Not that a few studies have been done about the effects of the TiO_2 nanoparticles on the male sexual organs and productivity potential.

This study investigated the effects of intraperitoneally injection of TiO_2 nanoparticles at different dose (30 and 50 mg/kg) on the testes tissue and male sex hormones.

MATERIALS AND METHODS

Nano titanium dioxide (TiO_2) particles prepared from Neutrino Company (Spanish). XRD results showed nano TiO_2 is in crystalline phase with 18 nm size. Purification of nano TiO_2 determined as 99.986 % by ICP-MS. Table 1 summarizes features of nano TiO_2 used in present study.

Table 1: Physical parameters of nano TiO_2 used in present study

Color	With
Morphology	Spherical
Crystallinephase	78/8% anatase,21/2% rutile
Specificsurfacearea	100-150 M^2/g
Density	3.84 gcc
Size	18 nm
purity	99.986 %

These features are more important in chemical and biological properties of TiO_2 nanoparticles

2.1. Animals

This study is an experimental effort that carried out on animals and we used adult male Wistar rats weighing 150-250 g were estimated from the animal house of martyr portal was developed. Animals whit average age of 2 months selected. Testing carried out at temperature of 20-25 centigrade degree that day duration was 12 hours and 12 hours dark lighting. Municipal tap water was used adjusted drinking water and eating animals for food by rats (feed compression) that the company prepared feed was barking in this study. Experimental animals were randomly divided into 4 groups (8 rats in each group) as follows: fist control group feed by usual water and food. Second control group that referred to Placebo, injected by 1 ml distillated water every other day intraperitoneally for equivalency of shock that obtained by intraperitoneally injection. Other groups from 3rd and 4th injected by 1 ml TiO_2 nanoparticles in 30 and 50 mg/kg doses, injection repeated every other day intraperitoneally. This continued until 21 day. TiO_2 nanoparticles resolved in physiological serum in 20 min by sonication method to producing a stable suspension.

One day after the last injection, blood sample of all animals prepared from neck veins. After clotting, samples were centrifuged at 3000 rpm for 15 minutes. After separating the serum from the clot by Smplr, serums frozen at temperature of - 20 ° C and stored, then used for hormones measurement. LH, FSH and testosterone hormones measured by Immuno radiometric assay.

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

2.2. Histological examination

One day after the last injection after blood sampling, rats anesthetized the testes separated and were immersed in 10% formalin. Samples were divided into 5 micrometer and stained by hematoxylin and eosin method and studied by light microscope.

RESULTS

3.1. The weight changes of rats in different groups

Injection of TiO_2intraperitoneallymay be causes physiological changes such as changes of weight in treated rats. Results analyzed by T-test were the difference in the level P <0.05 was considered significant. Rats weighing results showed no significant increase as table 2. Weighing results are in the table 2.

Table 2: the weighting results of rats before of injection and blooding

Groups	weight before injection (gr)	weight before blooding (gr)
Control group	251.37±125.17	213.50±27.86
Placebo group	224.12 ±49.91	228.25±29.38
Treated by 30 mg/kg of dose	226.87 ±18.69	226.75±15.69
Treated by 50 mg/kg of dose	236.87 ± 28.14	232.25±20.13

3.2. Sexual hormones concentration and response to TiO_2 treatment

After 11 injections of TiO_2 nanoparticle intraperitoneally, blood sample prepared and blood serum separated. After that sexual hormone concentration such as LH, FSH and testosterone measured. Results showed LH hormone level significantly increased and Testosterone decreased in TiO_2 treated group rather than control and placebo group (P<0.001). while the FSH hormone level doesn't have any significant change than control and placebo (P= 0.05).Hormone concentration results come as table 3.

***Table 3*: Results of LH. FSH and testosterone concentration in blood serum**

Groups	LH (mui /ml)	FSH (mui /ml)	Testosterone (ng/ml)
Control	0.10±0.037	0.10±0.00	1.80±0.54
Placebo	0.09 ±0.005	0.09± 0.005	1.42 ±0.77
30 mg/kg dose	1.32 ±0.777	0.10±0.00	0.44±0.628
50 mg/kg dose	3.10±0.625	0.10± 0.00	0.45±0.37
	P<0/001	P>0/05	P<0/001

Testosterone decreased and LH increased but FSH doesn't show significant change.

3.3. The results of histological studies of TiO_2 treated rats

Testicular tissue pieces that stained by hematoxylin and eosin and studied by light microscope. Comparison of figure 1A and 1B showed density of reproductive cells (spermatogonia), spermatocytes and spermatids and mature sperm did not significantly differ in groups that treated by 30 and 50 mg/kg dose of TiO_2 nanoparticle rather than the control and Placebo groups. Therefore eleven series of intraperitoneally injections with titanium dioxide nanoparticles by dose of 30 mg and 50 mg per kg created no disturbance in the testes tissue.

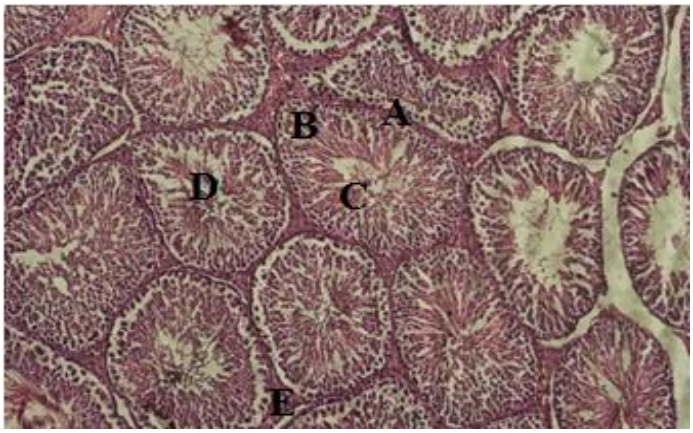

Fig 1A

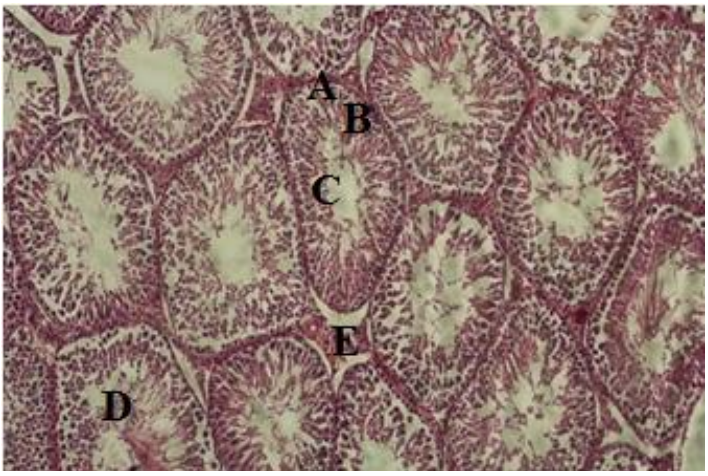

Fig 1B

Figure 1: histological studies on testis tissue showed no significant changes in TiO_2 treated rats rather than controls. Figure 1A related to control and 1B related to treated rat. A referred to spermatogonia, B is Spermatocytes, C is spermatid, D and E referred to immature sperm and Leydig cells respectively.

DISCUSSION

Nanoparticles have very specific chemical and physical characteristics of size, shape and high surface area to volume ratio that facilitate its medical and biological applications. This material distributed in all of body rapidly after injection by circulation and reached to the all of the organs and tissues (Berry et al. 2004). Before its application as medicine equipment, effects of nanoparticle on environment, biocompatibility and its toxic effects on human and animals should be assessed. These particles because of small size have high surface area and they are highly reactive, it is one important reason for its toxic effects (Carlson et al. 2008). TiO_2 nanoparticle due to optical, electrical and catalytic properties, have very important applications in various industries, including industrial pigments, sun block, bioremediation, air and water filtration and cancer treatment (Mital et al. 2011). Therefore because of widespread use of TiO_2 nanoparticle human and animal are exposing to this material. Aim of our study is investigation of TiO_2 exposing effects in sex hormones concentration so its negative role in productivity.

Statistical Analysis of our results showed testosterone hormone concentration in TiO_2 treated rats significantly decreased rather than control groups ($P<0.001$). This could be caused by adverse effects of nanoparticles 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. Nanoparticles also increases the release of reactive oxygen molecules such as super oxidase and increased protein oxidation that it cause cell death therefore testosterone producing cells reduced (Carlson et al. 2008). Other hypothesis is decreasing of Steroidogenic acute regulatory star gene expression due to nanoparticle exposing caused reduced testosterone production. Product of this gene is a carrier protein that transports cholesterol to inner cell membrane of mitochondria for steroidal hormones production.

As results showed LH hormone level significantly ($P<0.001$) improved in treated rats than control and placebo. This improvement in rats that received50 mg/kg dose rather than 30 mg/kg group, so it is dose- dependent. But treated rats did not showed significant changes in FSH level than control and placebo ($P>0.05$). Increasing in LH level can be as a result of decreased testosterone by negative feedback. Reducing in testosterone cause rise in LHRH secretion from hypothalamus that is main result of LH increasing. Nanoparticles create increasing in cGMP by nitric oxide production that cGMP activate protein kinase G (PKG). PKG has important role in secretion of LHRH from hypothalamus so stimulates LH production and secretion indirectly (Colvin 2003, Lansdown 2007).

Previous study confirmed our results, Takeda confirmed intraperitoneally injection of TiO_2 nanoparticle deteriorate reproductive system of male rats by decrease in sperm count and motility, increased sperm abnormalities and apoptosis of germ cells. But not reported any certain pathological changes in the testis and epididymis tissues

(Takeda et al. 2009). Other study showed hypodermic injection of TiO_2 nanoparticles cause accumulation of these materials in rat's testis (Included in Leydig cells, Sertoli and spermatid) (Gou et al. 2009).

Ema in 2010 confirmed rats that exposed to TiO_2 nanoparticle showed reduction in daily sperm production and sperm mobility and abnormality in sertoli cells (Emaet al. 2010).

Not that there are slight differences in our results and previous studies that is due to the change in coating, shape of crystal and nanoparticle size. Size is more important and in anatasecrystalline phase toxicity increased in smaller size (Morishige et al. 2010).

In the present study we have observed hunk of titanium dioxide nanoparticles in the abdominal cavity in treated rat dissection that it can be a reasons for non-toxic or low toxic effects of TiO_2 on testis tissue. Most of nanoparticles modified as a result of interaction by organic molecules in animal body so they aggregate together and inactivated, because more of its feature like shape size and surface charge altered therefore biological feature such as penetrating ability changed and its toxicity reduced (Garcia et al. 2005).

CONCLUSION

Investigation of the nano-materials effects on workers' health, consumers, public health and the environment is more important because of development of nanotechnology and increasing industrial pollution and rise exposing of human to this materials. Our results showed injection of TiO_2 nanoparticles can disrupt proliferating system in male rats by decreasing in testosterone and increasing in LH hormone concentration (dose- dependent).

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