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**Research Article**

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# The effects of titanium oxide nanoparticles on testis tissue and spermatogenesis in mice

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**ABSTRACT**

*Titanium oxide nanoparticles with various capabilities such as high oxidation, biocompatibility, and photocatalytic features are used frequently in wide range of sciences including medicine, engineering, cosmetics production and pharmacology. Considering frequent use of titanium oxide nanoparticles, it is important to study the distribution and toxicity of it in body. The goal of this study was investigating the effects of titanium oxide nanoparticles on testis tissue of male mice. Forty male mice were divided into four groups: control group did not receive anything, placebo group, and two experimental groups which received 10 and 100ppm of nanoparticles via gavage. At the end of period testes were colored using hematoxylin eosin method and studied using light microscopy. Obtained data were analyzed using SPSS software. According to results, both 10 ppm and 100 ppm groups reduced testis stem cells and primary spermatocytes significantly which is in accordance to epididymis images. Titanium oxide nanoparticles can pass the blood-testis barrier, be accumulated in testis and damage testis. So this nanoparticle has negative effects on male sex.*

**Keywords**: titanium dioxide, Syrianmice, tissue, testis

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## INTRODUCTION

Materials in Nano scale are really important and the most important feature of them is surface increment in this scale. High surface to volume ratio in nanoparticles make them ideal for using in various categories including medicine. This has been considered lately in new technological based methods or drugs for curing hard diseases such as cancer [1]. On the other hand, matters in Nano sale are more toxic than normal size; they react faster and damage cells via oxidative stress mechanism. Titanium oxide nanoparticles are used now in products such as self-cleaning glasses, tiles and air or water purification filters [2].

These nanoparticles cause production of free radicals in skin cells and can damage DNA and make changes in proteins structure which may lead to tumor or cancer. Very specific properties of nanoparticles such as size, shape and high surface to volume ratio make them appropriate for medicinal and biological applications. These matters are distributed in organs and tissues rapidly after injection and are absorbed by cells highly [1].

Spermatozoa are produced in somniferous tubules. These tubules are constructed from coverage of a connective tissue layer, abase layer and one seminiferous epithelium. Fibrous tunica propria contains some fibroblast layers and covers seminiferous tubules. The innermost layer is attached to the basal layer is consisted from smooth muscle-like

cells which show smooth muscle properties. Sperm making epithelium tissue has two cell types: Sertolicells or supporting cells and cells that form sperm. These cells produce spermatozoa. Prior to using matters as medicinal tools, their biocompatibility or toxicity and their effects on biological environment of body must be studied. Harmful particles will be more reactive and toxic in smaller sizes. Inherent ratios of these particles cause more involvement per unit volume [3].

Titanium oxide nanoparticles have many important applications in various industries including pigments as photocatalysts for environmental cleanup, sunscreen creams, and water filtration and destroying cancer cells because of their specific light, electrical and catalytic properties [4].

This study was carried out to investigate the effects of titanium oxide nanoparticles on testis tissue and spermatogenesis of little laboratory mice.

## EXPERIMENTAL SECTION

Titanium oxide nanoparticles were prepared from Nano Sunny Company (Iran Nanotechnology).

XRD results showed that used nanoparticles were mainly anatase, crystal phase and about 20 nm.

Also, ICP-MS test showed +99% purity. It must be mentioned that for preparing $TiO_2$ solution, ultrasonic machine was used for 15 minutes.

Forty male mature mice from the age of 4-5 weeks and weight range of 25-30g were used. Sample were kept in animal nest of *Ostad Taher Research Center (Shahreza- Iran)* with free access to standard food, water and room situation (12:12 photo period,25$^{\circ}$C temperature and 25-30% humidity). Mice were kept for 7 days to adapt to environment.

Treatment groups were control group did not receive anything, placebo group, and two experimental groups which received 10 and 100ppm of nanoparticles via gavage. After separating, testes were kept in formalin10% and tissue was divided into segments with 5micro meter thickness. Segments were colored were colored using hematoxylin eosin method and studied using light microscopy.

Obtained data were analyzed using one way analysis of variance and Duncan test (95%) was used to compare means. Mean weights of mice were compared using paired t-test. All analysis were done using SPSS program.

## RESULTS AND DISCUSSION

Figure 1 shows the changes of stem cells in the cross-section of seminiferous tubules. According to graph, the number of stem cells of first and second experimental groups were significantly different from control group ($p<0.05$).

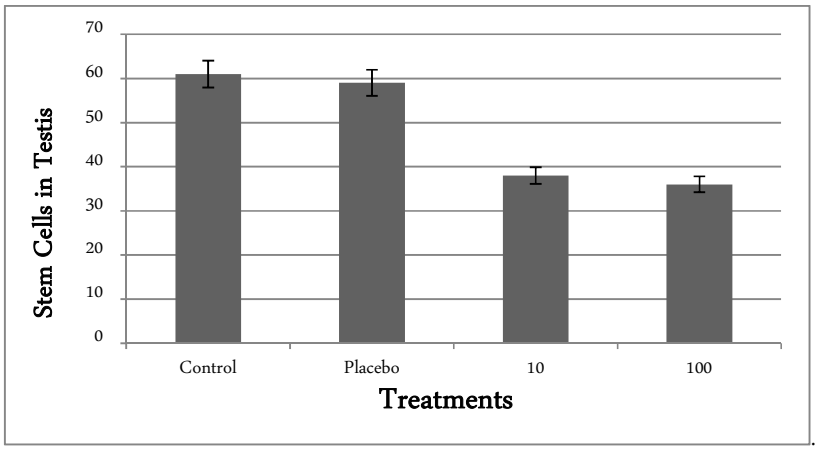


**Figure 1. The number of stem cells in treatment groups**

Figure 2 shows the changes of primary spermatocytes in the cross-section of seminiferous tubules. According to graph, the number of primary spermatocytes of both experimental groups were significantly different from control group ($p<0.05$).

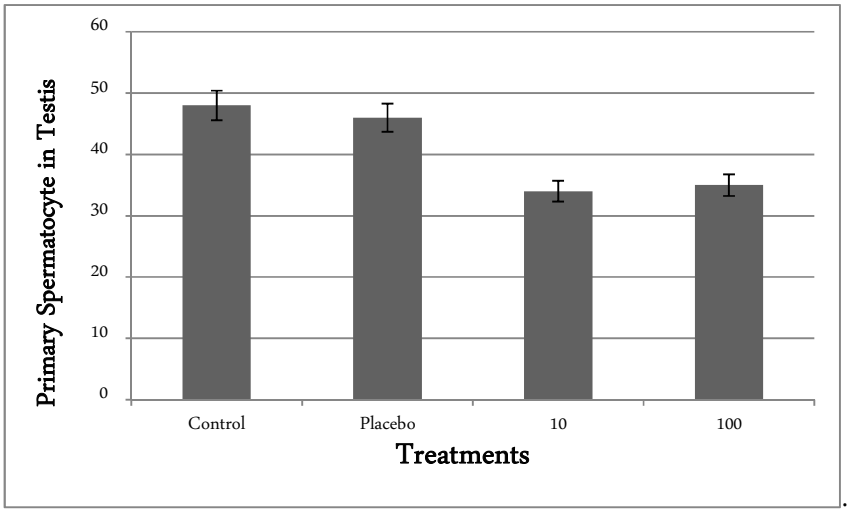


**Figure 2. The number of primary spermatocytes in treatment groups**

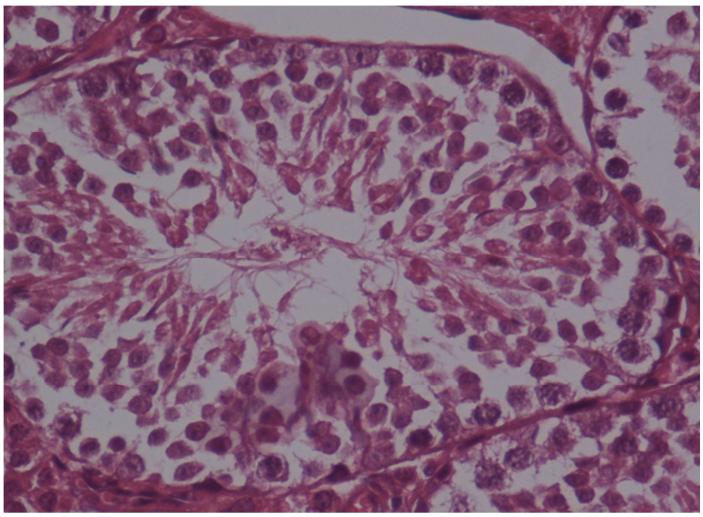

**Figure 3. Photomicrograph of seminiferous tubules in control group**

Accumulation of titanium oxide nanoparticles is an important factor for evaluating toxicity of reproduction system, but the way of entering nanoparticles to testis is not clear yet.

In this study, accumulation of nanoparticles in testis after gavage was evaluated for consequent 90 days. Nanoparticles passed blood testes barrier, were accumulated and led to reduce testicular mass, pathological changes in the testes and reduced sperm concentration, decreased sperm motility, increased concentration of abnormal sperms, sperm injury in long and narrow testicular rear appendages.

Also, intraperitoneal injections of 200 and 500 mg/kg of titanium oxide for seven days reduced motility and density of sperms highly, increased sperm abnormality and caused cell apoptosis in testis [1].

Intraperitoneal injections of $SiO_2$nanoparticles for 35 days (1.5 and 7.5 mg/kg) reduced the number of sperms, motility of sperms and increased abnormal sperms in rats considerably. Nanoparticles stimulated testicular injury

and prohibiting sperm formation in mice may be because of changes in levels of male sexual hormones and expression of testis genes.

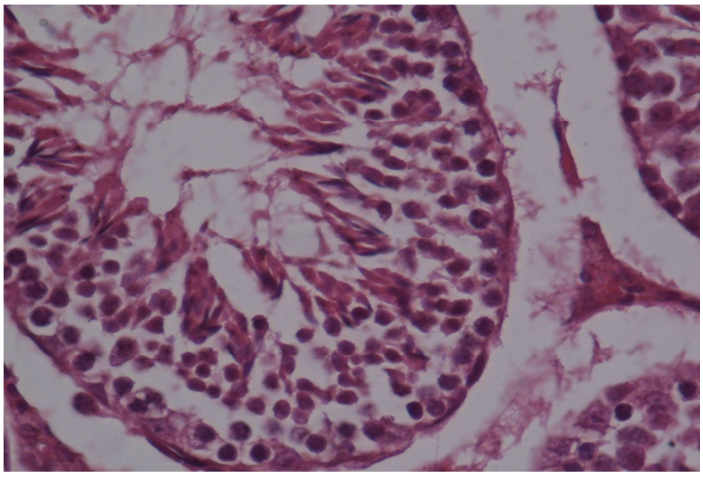

**Figure 4. Photomicrograph of seminiferous tubules in 10 ppm group**

Gao et al. studied hormones levels of male mice and evaluated harmony of all genes and found that long time exposing to titanium oxide nanoparticles changed expression (expression)level of 142 genes highly. Many genes are crucial for the formation of sperm and appear ina certain way of sexual cells or sperm producing stage [5].The last sperm production stage requires high dye density which is related to the shift of every small class of fundamental proteins. Genetic organ removing of transition proteins or organic chemical compound derived from fish sperm caused damages to the last step of the sperm creation [6].According to reports Adam3, spat19, and tdrd6 play main roles in sperm production process which Adam1 and Adam2 have accompanying activities and are used to guide adam3 to sperm membrane[7].Other researchers reported that removing adam3 gene damaged zona pellucida bond because adam3 in sperm population downstream of Adam network plays its role and does its duty in sperm zona pellucida binding [8].

Spata 19 is a specific testis protein which includes a mitochondrial targeting signal and work as an adhesion molecule between cover adjacent mitochondria and plays an important role in the final stage of spermatogenesis. ELIZA and qRT-PCR tests proposed titanium oxide exposing which led to reduced expression of Adam3, Spata19, and Tdrd in mice testis. Furthermore, we found that TiO2 exposing cause expression of Ly6e with 13906373 diff score considerably. Increased expression of Lyt6e was proven more by ELIZA and qRT-PCR rests. However it has been explained that expression of Ly6e does not have any effect on the development of the testes or sperm formation[7].

Therefore, reduced expression of prm1, Inp2, adam3, apata19 and Tdrd6 by exposing $TiO_2$nanoparticles causes probably reduction in sperm concentration in long narrow appendage of testis rear which includes sperm canals and causes the absence of sperm in the testicles. Male sexual hormones are very important. Increased serum concentrations of E2 and p4 plus reduced T, LH and FSH amounts were observed in male mice exposed to titanium oxide nanoparticles.

Actually, E2 affects steroid appearance directly in rats' testis [8].Increased E2 stimulates extra reproduction of rodents'leydig cells and is related to cryptorchidism, testis cancer and abnormal sperm[5].Furthermore, E2 has vital role in regulating the secretion of gonadotropin in male rodents and animals. FSH and LH are glycoprotein hormones which are secreted by front pituitary gland and act in testis directly to stimulate the performance of sexual cell tissue to support sperm production. Studies have shown that circulating FSH and LH concentrations have been reduced by E2 considerably [9].

## CONCLUSION

According to results, titanium oxide nanoparticles can enter testis via testicular blood barrier and be accumulated and cause testicular damage, sperm scar, and decreased sperm in long and narrow testicular rear appendages. Therefore, these nanoparticles have negative effects on male reproduction potential.

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