GSC Biological and Pharmaceutical Sciences, 2019, 06(01), 050–055

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(RESEARCH ARTICLE)

The effect of titanium dioxide nanoparticles injection in neonatal period on ovaries in mature rats

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Publication history: Received on 15 December 2018; revised on 10 January 2019; accepted on 14 January 2019

Article DOI: https://doi.org/10.30574/gscbps.2019.6.1.0160

Abstract

Titanium dioxide nanoparticles are one of the most widely used materials in various industrial and biological fields. In this study, due to the importance of reproduction in organisms, the toxicity of titanium oxide nanoparticles was investigated on ovaries. In this experimental study, concentrations of 50, 100 and 150 mg/kg titanium dioxide nanoparticles (TiO_2 NPs) with10-20 nm diameter were injected (IP) into immature female (age 35-40 days) for 5 consecutive days. Blood collection was performed three months after the last injection (at puberty) and the levels of hormones (LH, FSH, estrogen and progestron) in the serum were measured by ELISA. After anesthesia and dissection of animals, tissue sections of ovary were prepared and stained with hematoxylin-eosin. Then, the morphological status of ovarian tissue was investigated by optical microscopy. Data were analyzed using ANOVA. Results showed that weight of body and levels of LH and FSH in treated groups did not change significantly. Whereas, the amounts of estrogen and progesterone hormones increased significantly in the concentration of 150 mg/kg of TiO2 NPs. The TiO2 NPs caused histopathologic changes in the ovary including loss of Graafian follicles, destruction of follicles wall, reducing the thickness of Granulosa and Thec layers. Also, there was a significant decrease in the number of Corpus luteum, growing and Graafian follicles at concentrations of 100 and 150 mg/kg. It appears that injection of concentrations higher than 50mg/kg of TiO_2 NPs in the pre-pubertal period leads to impaired ovarian activity and structure after puberty, however further studies are needed to solidify fertility reduction in these treatments.

Keywords: Ovarian tissue; TiO_2 nanoparticles; Sex hormones

1. Introduction

Nanotechnology has been very influential in various fields of science and research and has led to significant advances in all sciences related to nanomaterials [1, 2]. Nanoparticles have more specific properties than larger sized materials, which make them effective in biological fields [3-5]. Due to their very small dimensions and highly reactive levels, they can easily pass through biological bariers and accumulate in the body organisms [6, 7]. Therefore, toxicity and side effects of nanoparticles are very likely on the environment and organisms [8, 9]. Research showed material at the nano size are more toxic than the large sizes materials, because they are highly reactive and cause oxidative stress in animals and human [10]. The toxicological effects of nanoparticles is still under investigation and their effects on biological systems incomplete [11, 5].

Titanium dioxide (TiO_2) is the world's first industrial product of nanomaterials in the world and have been widely used due to their strong catalytic activity and is used in pharmaceutical, cosmetics and paint industries. TiO_2 NPs can be absorbed in body by aspiration, ingestion and skin due to their small size [12, 5]. TiO_2 NPs are in different shapes, sizes, chemical compositions and have four crystalline polymorphic forms that anatase and rutile are the most common forms

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[13]. Its photocatalytic properties have been utilized to remove contaminants from water and air [14]. Various studies have been examined the effects of TiO_2 nanoparticles on different organisms [15, 16]. But since the most sensitive organs, especially in the neonatal period, are gonads and previous studies have often been reported on adults [17]. The purpose of this study was to investigate the toxicity of titanium dioxide nanoparticles in pre-puberty period on the pituitary gonad axis and oogenesis.

2. Material and methods

2.1 Preparation of TiO2 nanoparticles

TiO2 NPs with diameter of 10-20 nm and purity of 99.9% were prepared from Neutrino Company in Tehran of Province, Iran. The characteristics of nanoparticles used are based on previous research [16]. The 50, 100 and 150 mg/kg concentrations of the nanoparticles were prepared in normal saline, then, a relatively uniform suspension was prepared using ultrasonic waves (for 20 minutes, a temperature of 4 ° C and a range of 40%).

2.2 Animals

The immature female Wistar rats (40-50 g) were obtained from from Isfahan University of Medical Sciences. Animals were kept in laboratory conditions (20-23 ° C and natural light) for a week to adaptation. Different concentrations of TiO2 nanoparticles (50, 100, 150 mg/kg) were injected IP into three groups of treatment and normal saline into the control group (5 consecutive days) [18]. The animals were anesthetized and the blood samples were collected 3 months after the last injection of TiO2 NPs (adult rats). The hormones levels such as LH, FSH, estrogen and progesterone were assayed using autoanalyzer (Hitachi Automatic Analyzer, 17, Japan). Then, to observe the morphological changes of the ovarian tissue and the number of different follicles (Corpus luteum, growing and Graafian follicle) from each group, three rats were dissected after anesthesia and histological sections were prepared by staining hematoxylin-eosin (a total of 36 tissue lams) from their ovary. Histopathologic studies were performed by a microscope (Olympus CX22LED) equipped with a camera (Canon Eos 760D 18, Japan). Moreover, animals were weighed before and after treatment. This research was approved in advance by the department of biology, Falavarjan branch, Islamic Azad University Ethics Committee.

2.3 Statistical analysis

The mean of different factors were compared between treatment and control groups by one-way ANOVA and Duncan's tests (22 software).

3. Results

2.4 Hormonal changes

The comparison of weight changes in different groups showed that injection of TiO_2 nanoparticles did not affect animal weight gain (Table 1). Also, there was no significant change in the level of LH and FSH in the exposed groups of TiO_2 nanoparticles compared to control. Whereas, the amounts of estrogen and progesterone increased significantly in concentration of 150 mg/kg TiO2 NPs compared with other groups ($P<0.05$) (Table 2). On the other hand, the numbers of Growing follicles decreased significantly in groups exposed to TiO2 NPs 150 mg/kg concentration. Also, Graafian follicles decreased significantly in recipient groups of 100, 150 mg/kg TiO_2 NPs in a dose-dependent manner ($P<0.001$). Moreover, the numbers of Corpus Luteum decreased significantly in the treatment group of 100 mg/kg TiO_2 NPs) ($P<0.01$). In fact the numbers of Growing and Graafian follicles were correlated with TiO_2 NPs concentration (Table 3).

Table 1 Weight changes in rat after injection of TiO2 NPs

Group	Weight-1 (g)	Weight-2 (g)
Control	42.31±3.12	162.23±7.89
50 mg/kg TiO_2 NPs	45.40±2.46	173.25±6.13
100 mg/kg TiO_2 NPs	47.10±1.36	172.65±4.56
150 mg/kg TiO_2 NPs	43.15±2.15	169.37±4.35
P-value	P>0.05	P>0.05

Table 2 The effects of TiO_2 NPs injection on hormone levels

Hormone	Control	50 mg/kg TiO_2 NPs	100 mg/kg TiO_2 NPs	150 mg/kg TiO_2 NPs
LH (miu/ml)	0.82±0.67	0.54±0.27	0.56±0.36	0.78±0.33
FSH (miu/ml)	0.18±0.04	0.16±0.06	0.19±0.04	0.15±0.05
Estrogen (pg/ml)	63.79±13.78	66.31±26.27	93.70±41.50	114.83±52.27*
Progesterone (ng/ml)	4.64±2.08	3.82±1.05	2.81±1.05	6.82±2.15 ©

*Significant increase in the estrogen levels (P<0.05)
© Significant increase in the progesterone levels (P<0.05)

Table 3 The effects of TiO_2 NPs injection on follicles number of ovary

	Control	50 mg/kg TiO2 NPs	100 mg/kg TiO2 NPs	150 mg/kg TiO2 NPs	P value
Corpus luteum	11.83±1.32	12.66±1.75	8.66±1.21*	12.50±2.34	P<0.01
Growing follicle	9.66±1.63	11.33±2.25	11.33±2.16	7.50±1.87 ©	P<0.05
Graafian follicle	7.50±1.87	7.83±1.47	5.66±0.81 Ω	1.33±0.05 Ω	P<0.001

*Significant reduction in the number of Corpus luteum (P<0.01)
©Significant reduction in the number of Growing follicle (P<0.05)
Ω Significant reduction in the number of Graaf follicle (P<0.001)

2.4 Histological studies

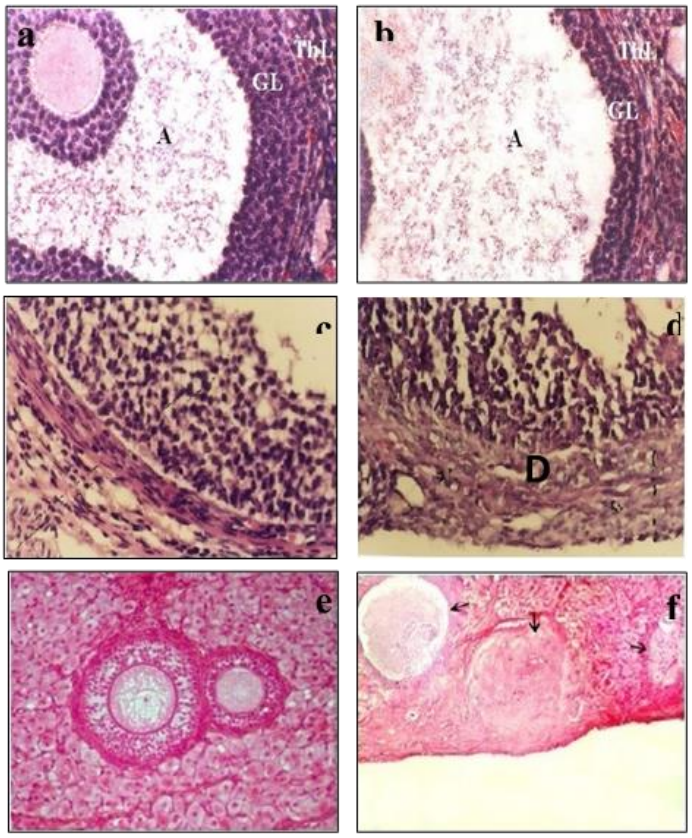

Figure 1 Sections of the ovary tissue in control and treated groups
(a) Graaf follicles in control group are normal, (b) treated group (150 mg/kg) shows loss of Graaf follicles and reducing the thickness of Granulosa and Thec layers, (c) growing follicles wall is regular in control group, (d) destruction of growing follicles wall in group 150 mg/kg (D), (e) follicles in control group are normal, (f) atretic follicles in group 150 mg/kg (arrows), (A, Follicular cavity; GL, Granulosa Layer; ThL, Thec Layer) (×400).

Histological studies showed changes, including loss of Graafian follicles and reducing the thickness of Granulosa and Thec layers in 150 mg/kg group compared with control group. Moreover, the destruction of growing follicles wall was observed in this group. Also, atretic follicle was observed in 100 and 150 mg/kg treatments of TiO2 NPs (Fig 1).

4. Discussion

Titanium dioxide nanoparticles have very special physical and chemical properties for use in medical and biological fields [16-18] and it is necessary that their side effects have been investigated on the organisms especially in immature animals. In the present study, intraperitoneal injection of titanium dioxide nanoparticles in immature female rats showed significant differences in a dose-dependent manner (at concentrations of 100 and 150 mg/kg) in the level of sex hormones and tissue structure of the ovary during puberty which included a significant increase in estrogen and progesterone levels, a decrease in the number of growing follicles, Corpus luteum and Graafian follicle. In general, because nanoparticles do not have a specific target tissue after entering the body [19, 20], precisely we can not justify the cause of these disorders. However, several studies similar to the present study have shown significant changes in levels of LH, FSH and sex hormones after treatment with TiO_2 nanoparticles [19-22], which have been reported to reduce fertility in some reports[19]. According to these researchers and other reports, the most common mechanism that the nanoparticles cause toxicity in the body, the production of free oxygen and hydrogen radicals consequently increased oxidative stress and ultimately by creating destructive effects on the membrane and cell structure in the various tissues that cause abnormalities in their function and structure [23-25]. Also, in several studies, the accumulation of titanium dioxide nanoparticles has been reported in subsequent injection in various organs such as gonads, which by binding to enzymes, different receptors, proteins, and even multiple genes, disrupting their function [20-22]. On the other hand, the probable accumulation of TiO_2 in various components of the reproductive system, the gonads and the gonad-pituitary axis, which is one of the most sensitive and complex physiological regulatory mechanisms, can cause severe fluctuations in the regulation of hormonal secretion [25, 26]. Other findings from the present study showed a decrease in the number of ovarian follicles with abnormal walls, which was associated with significant fluctuations in sex hormones levels, because the growth and activity of the follicles are influenced by these hormones and they have several positive and negative feedback effects on each other [23]. Additionally, in the current study, immature rats were exposed to TiO_2 nanoparticles, which the sensitivity of most organs and tissues, especially the gonads, is more than the period of puberty against various toxins including nanoparticles, because the gonadal development and the hormonal regulation pathways have not yet been completely developed [27]. Therefore, the time to use TiO_2 nanoparticles in this study is also one of the important reasons for the occurrence of hormonal and tissue disorders.

5. Conclusion

Our results showed that 5 times repeated injections of TiO_2 nanoparticles in pre-puberty at concentrations higher than 50mg/kg caused significant changes in sex hormones levels, number of follicles and their histological structure. But it can't be safely concluded that these changes lead to reduced fertility. Therefore, further research is needed to examine the fertility of rats exposed to titanium dioxide nanoparticles during pre-puberty.

Compliance with ethical standards

Acknowledgments

The authors would like to thank from the department of Biology, Islamic Azad University, Falavarjan Branch for their cooperation and support.

Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

This research was approved in advance by the department of biology, Falavarjan branch, Islamic Azad University Ethics Committee.

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How to cite this article

Noori A, Baghbadorani AA and Golozar M. (2019). The effect of titanium dioxide nanoparticles injection in neonatal period on ovaries in mature rats. GSC Biological and Pharmaceutical Sciences, 6(1), 50-55.