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Paper

PAPER

Inhibition of testosterone synthesis induced by oral TiO_2 NPs is associated with ROS-MAPK(ERK1/2)-StAR signaling pathway in SD rat

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Abstract

Titanium dioxide nanoparticles (TiO_2 NPs) have been widely used in food, medical, and other fields; their reproductive toxicity has been reported in numerous studies. However, the relevant toxicity mechanism still requires further exploration. In this paper, the effect of oral exposure to 500 mg/kg TiO_2 NPs (anatase and rutile) in adult male SD rats was studied over 3 and 7 days. Results showed that the total sperm count and testosterone level of 7 days of exposure in serum decreased in the experimental group. Testicular tissue lesions, such as disappearance of Leydig cells, disorder of arrangement of spermatogenic cells in the lumen of convoluted seminiferous tubules, and disorder of arrangement of germ cells, were observed. Meanwhile, the expression of steroidogenic acute regulatory (StAR; the key factors of testosterone synthesis), MAPK (ERK1/2), and phosphorylated ERK1/2 in testes of SD rats after exposure to TiO_2 NPs for 7 days decreased, while the malondialdehyde content increased and superoxide dismutase activity decreased in serum. The present study showed that TiO_2 NPs could cause reproductive toxicity. Notably, anatase is more toxic than rutile. In addition, exposure to 500 mg/kg TiO_2 NPs for 7 days inhibited testosterone synthesis in male rat, which may be related to the reactive oxygen species (ROS)-MAPK (ERK1/2)-StAR signal pathway. Warning that the use of TiO_2 NPs should be regulated.

Highlights

- Titanium dioxide nanoparticles (TiO_2 NPs) induced reproductive toxicity in the SD rats.
- Inhibition of MAPK (ERK1/2) expression of ROS induced by TiO_2 NPs.
- Inhibition of testosterone synthesis induced by oral TiO_2 NPs is associated with ROS-MAPK(ERK1/2)-StAR signaling pathway in SD rats.

Key words: titanium dioxide nanoparticle, smale reproductive toxicity, reactive oxygen species, ERK1/2, testosterone synthesis

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Introduction

In recent years, nanomaterials have been widely used in various fields, especially in food additives and food contact materials [1]. Nano-silver, titanium dioxide, and silicon dioxide are the most commonly mentioned nanomaterials in literature [2]. Titanium dioxide nanoparticles (TiO_2 NPs) is one of the most widely used nanomaterials in varying fields, including medicine [3], agriculture [4], bacteriostasis [5], wastewater treatment [6], personal care products [7], cosmetics, sunscreen, toothpaste, paint, and food [8]. TiO_2 NPs are inevitably released into the environment due to widespread use, which will harm individual organisms and ecosystems. TiO_2 NPs mainly exist in three different natural crystal forms, namely, anatase, rutile, and brookite [9]. Anatase and rutile are more commonly used than brookite; moreover, studies have reported that the surface activities of anatase and rutile differ [10]. Thus, these two kinds of crystal materials were selected for this study. Given the wide range of uses and special physical properties (small size), TiO_2 NPs are transferred to the human body through inhalation, environmental intake, and medical applications (skin exposure) [11, 12] and then enter different organs through blood circulation, such as the heart, liver, lung, brain, and testis [13, 14]. According to previous studies, after continuous exposure for 14 days, the deposition of TiO_2 NPs in various organs of mice is in the following order: liver > kidney > spleen > lung > brain > heart [15]. TiO_2 NPs enter the testis through the blood–testis barrier (BTB) due to their small size and then migrate to the testicular microenvironment composed of Sertoli cells, sperm cells, and Leydig cells (LCs). They then destroy the normal structure of the testis [16, 17] and inhibit testosterone production; lead to a decline in sperm quality; and cause a decrease in LCs and sperm count, sperm motility, and other male fertility problems [18, 19]. However, studies on the mechanism of TiO_2 NPs inhibiting testosterone production are few.

The process of testosterone synthesis has been reported [20]. First, cholesterol is carried from the outer membrane of the mitochondria to the intima through steroidogenic acute regulatory (*StAR*), and its side chain is cleaved and converted into pregnenolone by cytochrome P450 family 11 (*Cyp11a1*) in LCs [21]. Pregnenolone is transported to the smooth endoplasmic reticulum in the cytoplasm and forms testosterone through the reaction catalyzed by 3β-hydroxysteroid dehydrogenase ($3\beta HSD$) and $17\beta HSD$ [22, 23]. *StAR* is an acute regulatory protein [24], which regulates the transfer of cholesterol to the mitochondria. Otherwise, it is considered a rate-limiting step in testosterone synthesis and is regulated by extracellular regulated protein kinase 1/2 (ERK1/2) [25, 26]. More specifically, the upstream of *StAR* gene expression is regulated by phosphorylated ERK1/2 (Only phosphorylated ERK1/2 is active) [27]. *ERK1/2*, a member of the mitogen-activated protein kinase (MAPK) family [28], has been shown to promote the transcription of the *StAR* gene and may be involved in testosterone synthesis [29, 30]. In addition, previous studies have shown that MAPK is activated by reactive oxygen species (ROS) [31, 32], which may affect the normal expression of MAPK (*ERK1/2*) in the testis and affect testosterone synthesis. Notably, a large number of studies proved that the entry of TiO_2 NPs into the body may break the oxidation balance and generate ROS, resulting in a series of adverse effects, such as hepatotoxicity, reproductive toxicity, and genotoxicity [33, 34].

Here, we hypothesized that oral exposure to TiO_2 NPs in male rats may lead to the production of ROS and further activation of MAPK (*ERK1/2*) by ROS. *ERK* and *StAR* regulate each other [35]. Interference with the expression of *ERK1/2* will lead to the inability of *StAR* to transport cholesterol to the mitochondria normally, resulting in the inhibition of testosterone synthesis in the testis.

In this study, the adverse effects of anatase and rutile TiO_2 NPs on male reproductive function were evaluated by analyzing testicular histopathology, sperm count, and testosterone levels. The potential mechanism underlying the decreased testosterone synthesis pathway induced by TiO_2 NPs was investigated through 3 and 7 days of oral exposure in male rats.

Materials and Methods

Characterization of TiO_2 NPs

TiO_2 NPs of anatase and rutile types (40 nm ± 5 nm) were obtained from Aladdin industrial Corporation (Shanghai, China). The size of TiO_2 NPs was characterized by emission scanning electron microscopy (SEM) (JSM 6701F, JEOL Ltd, USA) after being dispersed by anhydrous ethanol and more than 100 nanoparticles sizes were measured by Image J software.

Animals treatment

Approximately 8-week-old adult male Sprague Dawley (SD) rats were purchased from the experimental animal center of Nanchang University (Nanchang, China). And then, the animals were kept in non-toxic and harmless cages in a room with a temperature at 22 ± 2°C and humidity of 45 ± 5% and 12 h cycle of dark and light. Animals have free access to clean drinking water and commercial food. Before the end of the experiment, clean the cage every 3 days in order to maintain a comfortable environment. The animal trials program was carried out with reference to the guidelines for experimental animal welfare and approved by the animal care review committee (approval No. 0064257), Nanchang University.

Adapting for a week, the animals were randomly divided into control, anatase, and rutile groups ($n = 5$). The study of Ali showed that exposure to 500 mg/kg TiO_2 NPs had oxidative damage in mice [36]. The rats in the experimental group were oral administered with 500 mg/kg TiO_2 NPs for 3 and 7 days in this study. Meanwhile, the control group was fed with the same amount of solvent. The clinical symptoms of rats were observed every day during the experiment. Twenty-four hours after the last administration, serum and testicular tissue samples of rats were obtained and stored in the refrigerator at −80°C.

Sperm counts

The assessment of testicular sperm count was adopted from the studies reported by Kisin *et al*. [37], and we made minor modifications. The isolated left epididymis was completely cut up and then put into 1 ml of saline and incubated at 37°C for 15 min in order to fully dissociate the sperm. 200 μl sperm sample was killed with hot water at 90°C, and then 10 μl sample was put into the blood cell counter to observe and determine the sperm count under light microscope (Nikon eclipse Ti). All studies were in triplicate.

Assay for testosterone in serum

The effects of TiO_2 NPs exposure over 3 and 7 days on the testosterone levels in serum were evaluated. According to the

instructions provided by the manufacturer, we used ELISA kit (Item No. 582701) to determine the content of testosterone in serum samples. Absorbance was recorded between 405 and 420 nm with the microplate reader (Thermo Scientific, USA). The plate should be read when the absorbance of the B_0 wells is in the range of 0.3–1.0 A.U (blank subtracted). The accurate testosterone content was calculated according to the standard curve.

Histopathology examination

Histopathological damage of testis exposed to TiO_2 NPs was evaluated. The testes of rat were extracted and stored in Bonn fixed solution for 24 h, then 75% alcohol was replaced for storage. The paraffin blocks were obtained by embedding the sample in paraffin after dehydration of ethanol and transparency of xylene, which were cut at 5 μm thickness and stained with hematoxylin and eosin. The stained sections were fixed on glass slides and magnified 200 and 400 times, respectively, under optical microscope to observe the seminiferous tubules, lumen, interstitial area, and germ cells.

Real-time quantitative polymerase chain reaction

According to Axyprep TM Multisource Total RNA Miniprep kit instructions (Takara Bio Inc.), total RNA was extracted from testis and reverse transcribed into cDNA. The primers were designed with NCBI Primer and Oligo Primer Analysis Software version7.0 (Molecular Biology Insights, Inc.; DBA Oligo, Inc.). And then, the primers were synthesized by Qingke Biology Co, Ltd (Shanghai, China). In the AriaMx Real-time quantitative polymerase chain reaction (RT-qPCR) system (MY 19435252), we used the three-step method, that is 1 min at 95°C followed by 40 cycles of 95°C for 5 s, 59°C for 60s, 72°C for 30 s. The relative quantification of mRNA was calculated by $2^{-\triangle\triangle Ct}$ method and GAPDH was used as the internal reference gene.

Immunohistochemistry (IHC) analysis

Paraffin sections of testicular tissue were dewaxed with xylene and ethanol and then placed in citric acid antigen repair buffer (pH 6.0) to repair antigens. The sections were incubated in 3% hydrogen peroxide and kept away from light at room temperature for 25 min in order to block the endogenous peroxidase. Next, add 3% BSA covering tissue and seal at room temperature for 30 min. After dripping primary antibody against StAR and pERK1/2 (1:500) and incubating overnight at 4°C, and adding corresponding secondary antibody (HRP marked, Servicebio, GB23303) for 50 min at room temperature. Finally, the sections were developed with diaminobenzidine (DAB). IHC was quantified with reference to the previous method and quantified with Image J software. The results obtained were converted into scores using $[\sum Pi(i+1)]$ formula (i: staining intensity score; Pi: percentage of stained contribution, negative: 0, low positive: 1, positive: 2, high positive: 3).

Detection of superoxide dismutase, catalase and malondialdehyde

The activities of superoxide dismutase (SOD) and catalase (CAT) as well as the content of malondialdehyde (MDA) in serum were measured according to the instructions of the kit manufacturer, which purchased from Jiancheng Bio-tech Co. Ltd (Nanjing, China).

Statistical analysis

All the reported data were displayed with mean ± standard deviation (SD), which was analyzed using the SPSS (version 22) software. Differences between groups were analyzed by one-way analysis of variance. Significant differences were expressed by * (*$P < 0.05$,**$P < 0.01$,***$P < 0.001$). Specially, at least three independent parallel experiments were conducted in each group to ensure the authenticity of the reported data.

Results

Characterization of TiO_2 NPs

To explore the dispersion of nanoparticles in solution and the true particle size and shape, this study was observed by SEM. The results showed that the two types of TiO_2 NPs were spherical with an average diameter of anatase of 37 nm and rutile 46 nm (Fig. 1).

Sperm counts

After exposure to different crystalline TiO_2 NPs for 3 and 7 days, the spermatozoa of the left epididymis were collected and counted with the blood cell count plate. Compared with the control group, the total sperm count of the anatase and rutile groups after oral administration for 3 (Fig. 2A) and 7 days (Fig. 2B) decreased significantly ($P < 0.001$). In addition, the total sperm count of the anatase group was significantly lower than that of the rutile group ($P < 0.05$). The results showed that acute exposure to different crystal forms of TiO_2 NPs had adverse effects on sperm survival in the testes of SD male rats, and the effect of anatase was greater than that of rutile.

Histopathological evaluation

The histopathological evaluations of the testicular sections and histomorphology of the control group revealed normal findings. The seminiferous tubules were closely connected and arranged neatly; the interstitial region was intact; germ cells were abundant in the control group; and spermatogonia, spermatocytes, and sperm cells were clearly observed (Fig. 3A-a and B-a). In the anatase group exposed for 3 (Fig. 3A-b) and 7 days (Fig. 3B-b), the number of LCs disappeared; spermatogenic disorder appeared in the lumen of seminiferous tubules; interestingly, germ cells were randomly arranged, the number decreased, and spermatocytes were exfoliated and vacuolated. The pathological condition in the rutile group was similar to that in the anatase group, but Fig. 3A-c and B-c shows that the pathological condition was not poor. Therefore, acute exposure to TiO_2 NPs could cause damage to the testis of animals and interfere with the production of spermatozoa.

Testosterone levels

The change in the testosterone level determined by an ELISA kit is shown in Fig. 4. The results showed that the testosterone levels in serum exposed for 7 days were lower in the treatment group than in the control group, and the anatase levels were lower

Figure 1: Characterization of TiO_2 NPs. (A, C) SEM image of anatase and rutile TiO_2 NPs. (B, D) The frequency of the size distribution of anatase and rutile TiO_2 NPs.

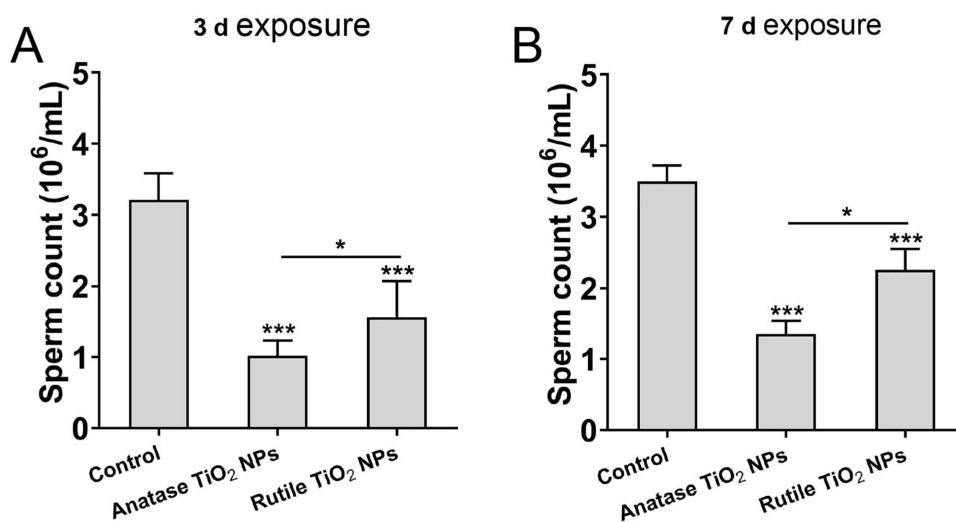

Figure 2: The number of sperms in the left epididymis of male rat after oral administration of 40 nm TiO_2 NPs. The data are presented in the form of sperm per milliliter of saline. (A) 3-day exposure, (B) 7-day exposure. Data are expressed as mean ± SD. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with control.

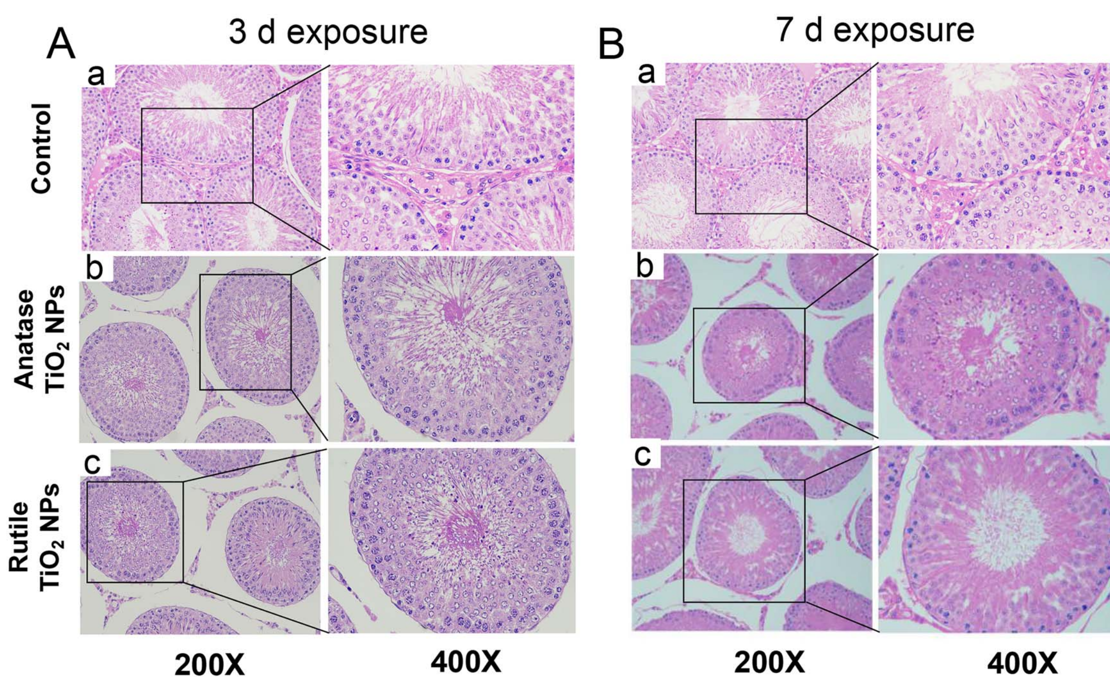

Figure 3: Light microscopy of cross sections of hematoxylin and eosin-stained testes from male rat. (**A**) 3-day exposure, (**B**) 7-day exposure.

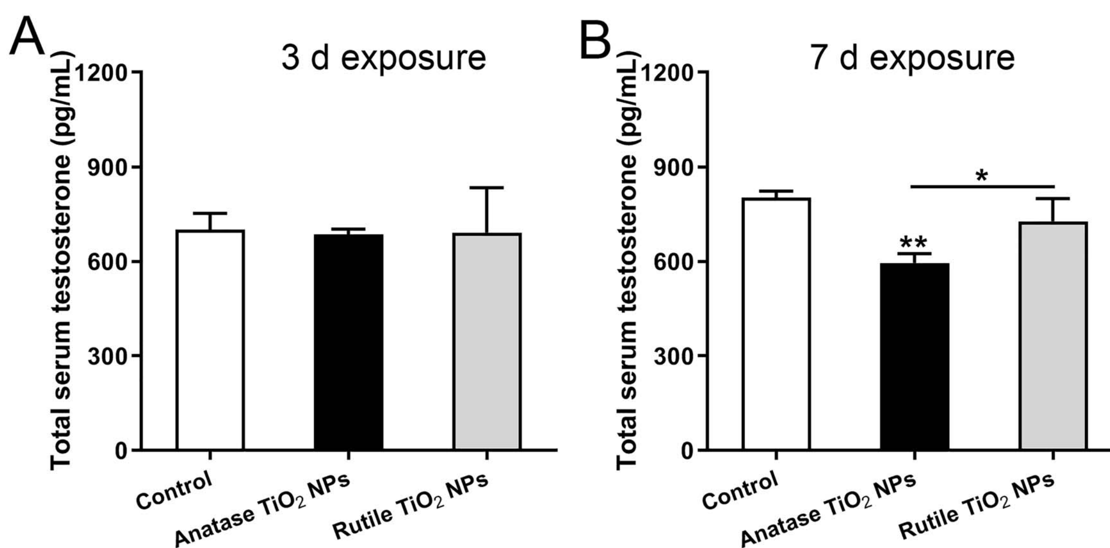

Figure 4: Testosterone concentration in male rat serum detected by ELISA. (**A**) 3-day exposure (**B**) 7-day exposure. Data are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control.

in the treatment group than in the rutile group. These findings indicated that acute exposure to different crystal forms of TiO_2 NPs could affect the synthesis of testosterone, and the effect of anatase was more serious than that of rutile.

Change in relative gene quantification in testis

In this study, the changes in gene quantification related to ROS, MAPK (*ERK1/2*), and testosterone production were assessed by RT-qPCR. The primer information of related genes is shown in Table 1. In the 3-day exposure group, the expression levels of ROS-associated gene of nuclear factor erythroid 2-related factor 2 (*Nrf2*), NAD(P)H quinone dehydrogenase 1 (*NQO1*), glutathione (*GSH*), *CAT,* superoxide dismutase 2 (*SOD2*), and MAPK (*ERK1/2*) were significantly downregulated in the experimental groups compared with the control groups. The testosterone production-associated gene of *StAR* was markedly upregulated in the experimental groups. By contrast, *Cyp11a1* was markedly downregulated. 3βHSD was significantly downregulated in the anatase group and upregulated in the rutile group, whereas *17βHSD* was significantly upregulated in the anatase group and downregulated in the rutile group. Similarly, in the 7-day exposure group, the ROS-associated gene of *GSH* was significantly upregulated, whereas *HO-1* was downregulated in the experimental groups. *CAT* and *NQO1* were downregulated in the rutile group, while *Nrf2* showed no significant alteration. MAPK (*ERK1/2*) was significantly downregulated in the anatase groups. The testosterone

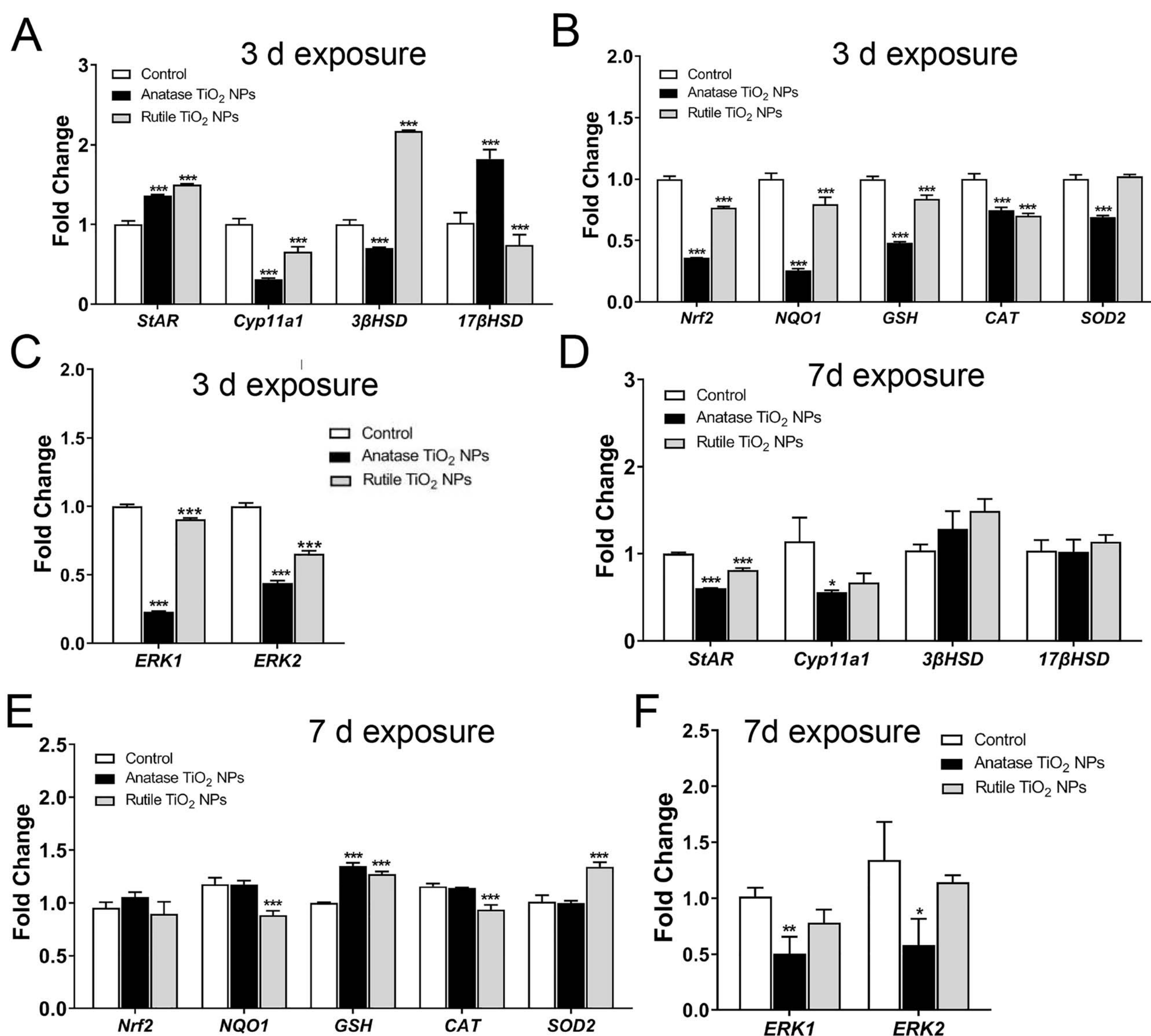

Figure 5: Gene expression of testosterone synthesis, ROS, and MAPK (*ERK1/2*) in testis. (**A–C**) 3-day exposure, (**D–F**) 7-day exposure. Data are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control.

Table 1: Sequences of the primers used for quantitative real-time PCR

Target gene	Forward primer (5′-3′)	Revere primer (3′-5′)
StAR	TCCTCGCTACGTTCAAGCTG	CGTCGAACTTGACCCATCCA
Cyp11a1	TAGCTTTGCCATGGGTCGAG	AGTACCGGAAGTGGGTGGTA
3βHSD	ACACGGCTTCTGTCATGGATT	CCAATAGGTTCTGGGTACCTTTC
17βHSD	TGCTTGGGTTTGGCACATT	TCTCTCCAGGCACTGACGTA
ERK1	AATGGAAGGGCTATGACCG	AGCTTGAGAGGGAGAGGGTT
ERK2	ATGACCCAAGTGATGAGCCC	GAGCCCTTGTCCTGACCAATTT
Nrf2	AGACAAACATTCAAGCCGAT	CTCTCCTGCGTATATCTCGAA
NQO1	TTGCTTTCAGTTTTCGCCTT	CCCCTAATCTGACCTCGTTC
GSH	ATCCCACTGCGCTCATGACC	AGCCAGCCATCACCAAGCC
CAT	ATAGCCAGAAGAGAAACCCACA	CCTCTCCATTCGCATTAACCAG
SOD2	ACTTGAAACGTGTAACTAGGC	CTTTCATACAATACACAGTCGG
GAPDH	TCCCTCAAGATTGTCAGCAA	AGATCCACAACGGATACATT

production-associated gene of *StAR* was significantly downregulated in the experimental groups. *Cyp11a1* was significantly downregulated in the anatase groups, while *3βHSD* and *17βHSD* showed no significant alteration (Fig. 5).

IHC analysis and ROS index determination

The results of IHC analysis of StAR and pERK1/2 in testes (Fig. 6A and B) showed that after 7 days of exposure to TiO_2 NPs, the experimental group decreased significantly, and anatase was more obvious than rutile. In addition, the MDA content in serum of the experimental groups increased significantly, and the anatase group increased more significantly than the rutile group (Fig. 6C). MDA is one of the most important products of membrane lipid peroxide. SOD activity decreased significantly in the anatase group; a downward trend was observed in the rutile group compared with that in the control group, but the difference was insignificant (Fig. 6D). Moreover, there was no significant difference in CAT activity among the groups, but it was observed that there was a decreasing trend in the experimental group, especially in the anatase group (Fig. 6E). These results suggested

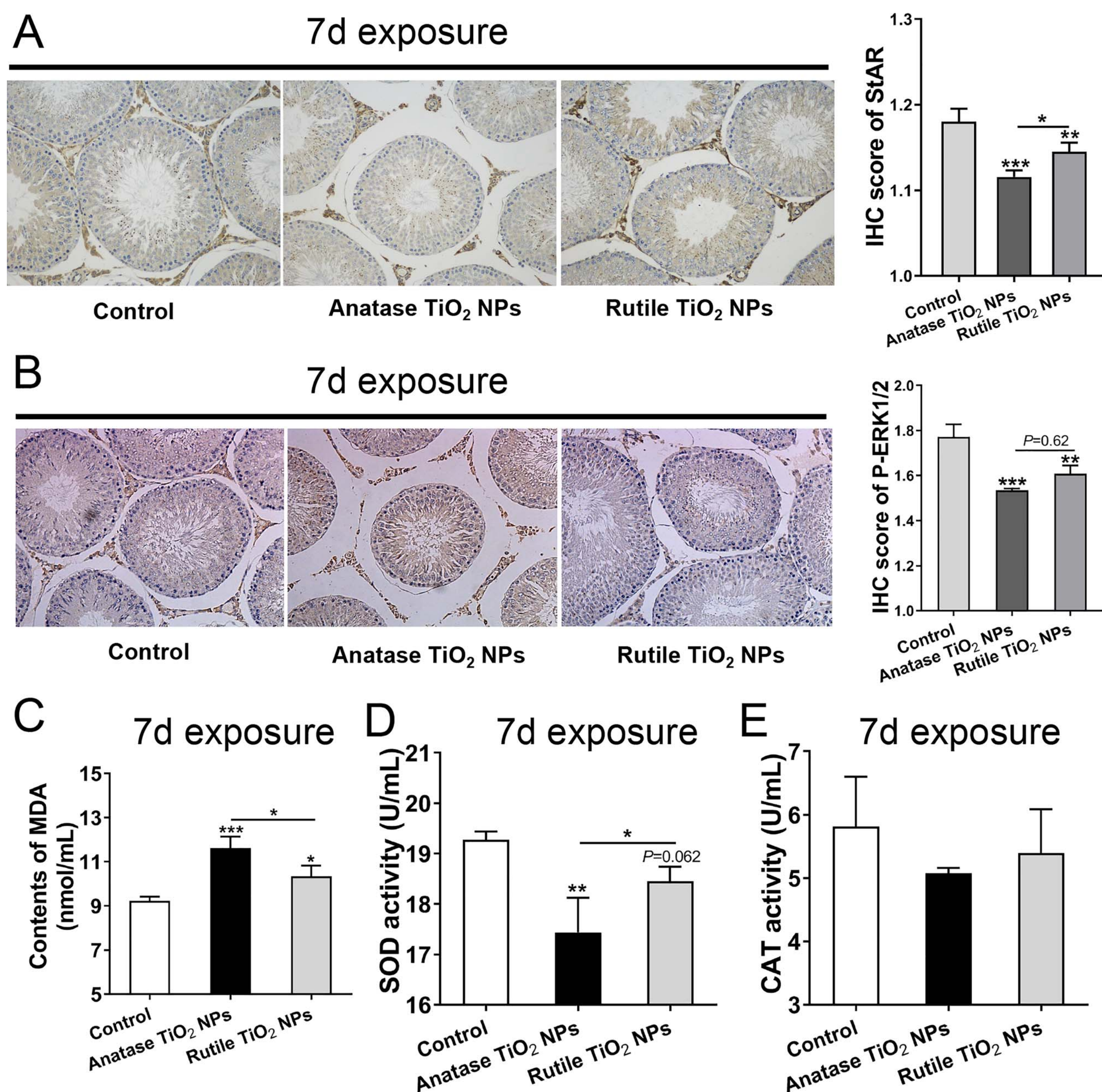

Figure 6: IHC analysis and ROS index determination. (**A**, **B**) IHC analysis of StAR and pERK1/2. (**C**–**E**) ROS index determination of MDA, SOD, and CAT in serum. Data are expressed as mean ± SD. *$P < 0.05$, **$P<0.01$, ***$P < 0.001$ compared with control.

that oral TiO_2 NPs for 7 days could induce ROS production and inhibit ERK1/2 phosphorylation and StAR expression.

Discussion

TiO_2 NPs inevitably enter the environment because they are widely used in different fields and enter the human body through different pathways, including oral administration, skin contact, and injections. Studies have shown that oral TiO_2 NPs are toxic to rodents and accumulate significantly over time due to slow tissue elimination and repeated exposure, notwithstanding low oral bioavailability [13]. Complementally, Hu *et al.* believes that it is easier to detect only higher doses of TiO_2 NPs with higher toxicity [38]. Male infertility is a global population health concern, and global rates of male infertility range from 2.5 to 12% [39]. Thus, studies on the reproductive toxicity caused by TiO_2 NPs are crucial. Results of this study clearly proved that oral exposure to anatase and rutile TiO_2 NPs (500 mg/kg) for 7 days decreased the content of testosterone in serum compared with the control group (Fig. 3). This finding was consistent with the results of Jia *et al.* [19], who reported that TiO_2 NPs can inhibit the synthesis of testosterone. The main organ of testosterone synthesis is the testis. The results of our histopathological sections showed that the normal structure of the testis was destroyed, such as seminal cavity disorder and shedding and vacuolation of germ cells. Moreover, a large number of LCs disappeared (Fig. 3). Similar results were reported by Elnagar *et al.* [40]. LCs are the main sites of testosterone synthesis and secretion; they are mainly

distributed in the loose connective tissue of seminiferous tubules [41, 42] and are responsible for the production of androgens to maintain normal male development and reproductive function [43, 44]. In addition, testosterone is the main component of androgen, which is transported to the target organs of the body and makes an important contribution to reproductive function by binding to receptors [45, 46]. Previous studies reported that TiO_2 NPs directly trigger androgen imbalance, which results in estrogen imbalance, testicular dysfunction, and inhibition of spermatogenesis [17]. In this study, the sperm count also showed similar results; the total number of sperm in the experimental group decreased considerably compared with that in the control group (Fig. 2). In general, TiO_2 NPs can cause testicular damage and affect testosterone levels. A decrease in testosterone levels can affect the testes and exacerbate abnormal testicular spermatogenesis. In other words, the reproductive dysfunction and decrease in serum testosterone level in male SD rats are closely related to TiO_2 NPs.

The synthesis and secretion of testosterone are regulated by the expression of *StAR, Cyp11a1, 3βHSD, 17βHSD*, and other genes [47]. In short, cholesterol is transported from the outer membrane of the mitochondria to the inner membrane of the mitochondria through StAR and is bioconverted to pregnenolone by *Cyp11a1*, which is then catalyzed by *3βHSD* and *17βHSD* in the endoplasmic reticulum to produce testosterone [48]. StAR is considered a rate-limiting enzyme in the transport of cholesterol to the mitochondria, where its expression is regulated by ERK1/2, and testosterone synthesis is directly affected by it [21, 49]. In the present study, the results showed that anatase TiO_2 NPs significantly downregulated the expression of the *ERK1/2, StAR*, and *Cyp11a1* genes in the 7-day exposure group (Fig. 4E and F). Similar results were obtained by Jia *et al.* [19] through oral administration of anatase TiO_2 NPs (10, 50, or 250 mg/kg) to mice. Interestingly, *StAR* was significantly upregulated (Fig. 4A) in the 3-day exposure group, which possibly contributed to the negative feedback regulation of the hormone synthesis pathway in short-term exposure.

ROS produced in the body has been proven to activate members of the MAPK family, which contains *ERK1/2* [50]. RT-qPCR results showed that ROS-related genes in the testis changed significantly after exposure to TiO_2 NPs. For example, after 3 days of exposure, the expression of the *GSH, CAT*, and *SOD2* antioxidant genes was significantly downregulated (Fig. 5B). Meena *et al.* [51] injected 50 mg/kg TiO_2 NPs (21 nm) intravenously in male Wistar rats; their results showed that antioxidant enzymes such as *CAT, GSH*, and *SOD* decreased significantly in the testis. In addition, the *Nrf2 and NQO1* genes related to the antioxidant pathway were significantly downregulated. TiO_2 NPs induced ROS production and damaged the antioxidant capacity in the body. The MDA content in serum increased while SOD activity decreased after 7 days of exposure (Fig. 6C and D). TiO_2 NPs destroy the oxidative balance in the testes of SD rats, which may be the source of the inhibition of testosterone synthesis.

Thus, the small size of TiO_2 NPs could reach the testis through the BTB and induce ROS production. ROS may directly lead to apoptosis, inhibit the expression of the *ERK1/2* gene, and reduce the phosphorylation level of *ERK1/2*, interfering with the normal transcription of the *StAR* gene. Finally, the synthesis of testosterone was inhibited (Fig. 7). Frankly, this study chose a high dose of TiO_2 NPs acute exposure to rats, which hardly reflect the exposure that animals will face in real scenarios. In that sense, it is meaningful to mention that these investigation provide experimental evidence for the potential risks of nanomaterials in daily use. However, it is necessary to study more realistic situations in future research, namely subchronic and chronic exposures, as well as low doses similar to what we could be exposed to with commercial nanotechnology-based products.

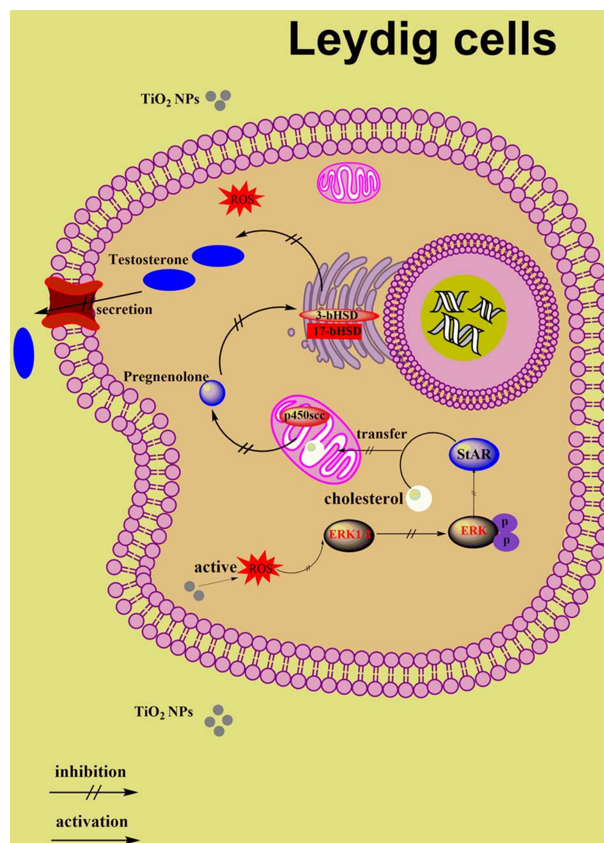

Figure 7: Schematic diagram for the suppression of testosterone production by TiO2-NPs associated with ROS-MAPK(ERK1/2)-StAR signaling pathway. Acute oral exposure to high doses of TiO_2 NPs in male rats would lead to the production of ROS and further activation of MAPK (ERK1/2) by ROS. ERK and StAR regulate each other, and interference with the expression of ERK1/2 will lead to the inability of StAR to transport cholesterol to the mitochondria normally, resulting in the inhibition of testosterone synthesis in the testis. ("↑" activation, "‡" inactivation, "P" phosphorylation)

Conclusion

The potential mechanism underlying oral exposure to different crystal forms of TiO_2 NPs for 3 and 7 days on testosterone synthesis in male SD rats was explored. The results showed that exposure to TiO_2 NPs was harmful to sperm production and inhibited the synthesis of testosterone. The destruction of testicular tissue was manifested by the disappearance of LCs and exfoliation and vacuolation of spermatogenic cells. In general, the results after exposure to anatase for 7 days were more significant than those after exposure to rutile. Thus, anatase led to greater toxicity than rutile, which may be attributed to the high surface activity of anatase [10]. RT-qPCR results showed that TiO_2 NPs inhibited the expression of antioxidant enzyme genes and induced ROS production in rats, which activated MAPK (ERK1/2). The expression of *ERK1/2* and *StAR* was downregulated, resulting in the inhibition of testosterone synthesis. ROS-MAPK (*ERK1/2*)-*StAR* is a potential pathway in which testosterone synthesis is inhibited. Moreover, the synthesis and secretion of testosterone

are complex processes, and the compound dynamic route of TiO_2 NPs inhibiting testosterone synthesis needs to be further studied.

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Conflict of interest statement

The author reports no conflicts of interest in this work.

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