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RESEARCH ARTICLE

Protective effects of orally administered thymol against titanium dioxide nanoparticle–induced testicular damage

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Abstract

In this study, we investigated the potential of thymol and its mode of action to protect against the titanium dioxide (TiO_2) nanoparticle–induced testicular damage. Twenty-four rats were randomly divided into four groups: control group, TiO_2 (100 mg/kg BW/day) group, TiO_2 + thymol (10 mg/kg BW/day) group, and TiO_2 + thymol (30 mg/kg BW/day) group. With the exception of the control group, all animals received orally TiO_2 nanoparticles for 60 days. In treatment groups, animals were given orally thymol 1 h before TiO_2 nanoparticles. Epididymal sperm parameters, testicular histopathology, and spermatogenesis assessments were performed for evaluation of the TiO_2 and thymol effects on the testis. Furthermore, antioxidative enzyme activities such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD), and malondialdehyde (MDA), glutathione (GSH) levels and ferric-reducing antioxidant power (FRAP) value were measured. Intragastric administration of TiO_2 for 60 consecutive days caused a significant decrease in sperm quality, widespread histopathological alteration, and significantly induced oxidative stress as manifested by elevated MDA levels and a remarkable decline in antioxidant enzyme activities such as CAT, SOD, and GPx, and also FRAP and GSH levels in testis tissue. Nearly all of these alterations were significantly ameliorated in the groups that orally received thymol before TiO_2 nanoparticles administration. The results of this study demonstrated that thymol improved the spermatogenesis defects induced by TiO_2 nanoparticles in rats in a dose-dependent manner by protecting the testes against the testicular toxicity. Reduction in TiO_2 nanoparticle–induced oxidative stress may have a major role in this protective effect.

Keywords Thymol · TiO_2 nanoparticles · Testis · Oxidative stress · Malondialdehyde · Spermatogenesis

Introduction

Today, the application of various synthetic nanoparticles is expanding and consequently enhancing environmental exposures to these materials. Among the various metal nanomaterials, TiO_2 nanoparticles are used as an additive in a wide variety of products such as food colorants, nutritional supplements, cosmetics, sunscreens, and toothpaste (Hong et al. 2015a; Weir et al. 2012). With the widespread application of TiO_2 nanoparticles, the concern about its cytotoxicity has been raised (Iavicoli et al. 2011). In vivo and in vitro investigations show that high doses of TiO_2 nanoparticles are accumulated in the testes and induce oxidative stress, inflammation, and apoptosis, which may lead to organ injury and failure (Alaee and Ilani 2017; Shakeel et al. 2016). Due to its special characteristics (such as the small size, high reactivity, and high surface area), TiO_2 nanoparticles are able to readily pass the blood-testis barrier and result in testicular histological abnormalities, sperm malformations, alterations in serum levels of sex hormone, etc. (Shakeel et al. 2016). According to previous studies, these nanoparticles, like many other xenobiotics, exert their toxic effects via inducing oxidative stress. Oxidative stress is usually defined as an imbalance

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between the production of free radicals and the ability of the biological system to counteract or detoxify their detrimental effects through neutralization by enzymatic (such as catalase and glutathione peroxidase) and non-enzymatic (such as glutathione) antioxidants (Alaee and Ilani 2017; Ghaffarian-Bahraman et al. 2014). Moreover, oxidative stress is able to induce inflammatory responses and mitochondrial dysfunction, leading to cell damage and eventually cell death in target tissues (Chen et al. 2008; Ghasemnejad-berenji et al. 2018a).

Due to the toxic effects of TiO_2 nanoparticles on testicular function following repeated and prolonged exposure, it seems crucial to find suitable agents for reducing their negative side effects on testis homeostasis (Orazizadeh et al. 2014).

In recent years, much attention has been paid to the beneficial effects of natural antioxidant compounds. Thymol is a natural monoterpenoid phenol obtained from the oils of thyme and plants such as *Thymus vulgaris*, *Thymus ciliates*, *Origanum vulgarae*, *Thymbra spicata*, and *Nigella sativa* seeds. Recently, it has been widely studied in different fields due to its multiple functional activities. Considerable amounts of data show that thymol has many beneficial biological effects including antibacterial, anti-inflammatory, antifungal, antimutagenic, analgesic, anticonvulsant, antiepileptogenic, antihemolytic, and radioprotective activities. The antioxidant activity of thymol has been reported in different experimental studies including animal models and cell lines. This activity is attributed to the presence of the phenolic hydroxyl group in its structure which can scavenge and neutralize free radical (Aboelwafa and Yousef 2015; Jafari et al. 2018; Meeran et al. 2017).

Since oxidative damage has been implicated in the toxic effects of TiO_2 nanoparticles on male reproductive system, the current study was conducted to investigate the probable protective effect of thymol on testis lipid peroxidation, non-enzymatic and enzymatic antioxidant levels in rats subchronically exposed to TiO_2 nanoparticles. Furthermore, the beneficial effects of thymol against TiO_2 nanoparticles were also evaluated by histological and sperm parameters.

Material and methods

Chemicals

General laboratory chemicals were obtained from Sigma-Aldrich (GmbH, Munich, Germany) unless otherwise stated. The nano-sized TiO_2 powder used in the present study was PC50 (manufactured by Cristal Global, 100% anatase, 40 nm primary particle size). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) commercial kits were supplied by Zell Bio GmbH Co. (Germany).

Animals

All male Wistar rats weighing between 180 and 200 g were obtained from the animal house of Medicine school, Urmia University of Medical Sciences (Urmia, Iran). Animals were kept in semi-transparent polycarbonate cages (15 cm × 27 cm × 43 cm) in a controlled room temperature (21 ± 5 °C), humidity (50–55%), and 12-h light/dark cycle. Rats were fed a complete rodent diet and acidified tap water ad libitum and deprived of food for 12 h prior to experiments. All experiments were done in compliance with the ethical guidelines on the use of animals and were approved by the Ethics Committee of Urmia University of Medical Sciences with code number IR.UMSU.REC.1398.217.

Study design

Twenty-four rats were randomly assigned to four groups of each six animals, as follows:

- Group 1 Animals received corn oil by gavage for 60 consecutive days and served as control group.
- Group 2 Animals received 100 mg/kg TiO_2 nanoparticles by gavage for 60 consecutive days and served as TiO_2 group.
- Group 3 Animals were given a daily oral dose 10 mg/kg thymol 1 h before TiO_2 nanoparticles at the same dose for 60 consecutive days and served as TiO_2 + thymol low dose.
- Group 4 Animals were given a daily oral dose 30 mg/kg thymol 1 h before TiO_2 nanoparticles at the same dose for 60 consecutive days and served as TiO_2+ thymol high dose.

TiO_2 nanoparticles were dispersed in distilled water, and then the suspending solutions were treated by ultrasonic for 20 min to make a homogeneous suspension. Thymol was dissolved in the corn oil, and the dose was selected according to the previous studies (Cardoso et al. 2016; Jafari et al. 2018).

Testis tissue preparation

At the end of the study, all animal were weighed, euthanized by overdose injection of ketamine. Then immediately, one testis from each rat within different treatment groups was separated, weighed, and homogenized in 4 volumes of phosphate buffer (pH 7.4). The homogenates were centrifuged using a cooling centrifuge at 4 °C, and the supernatant were frozen at − 20 °C in aliquots until used for biochemical evaluation. The caudal part of both epididymis was used for the determination of sperm parameters.

Coefficient of the testis and histopathological examination

After that, the body and the second testis of each animal was weighted, the coefficient of the testis to body weight was calculated as the ratio of tissue (wet weight, mg) to body weight (BW, g). Samples of testis tissues were taken and immersed in formalin 10% for histopathological examination. After routine tissue processing and embedding in paraffin, the 5 μm sections were stained with hematoxylin-eosin (H&E). The epithelial height and diameter of 10 round-shaped seminiferous tubules from each rat were randomly selected and reported. For this purpose, two diameters perpendicular to each other were measured in each seminiferous tubules, and their average was determined. To determine the average of the epithelial height of the same seminiferous tubules, we measured it from the basement membrane to the luminal surface at two locations. The measurements were performed using a light microscope equipped to micrometer lens at 100× magnification.

Biochemical analysis of testis tissue

To evaluate the activity of SOD, CAT, and GPx, a part of testis homogenate was centrifuged at 12,000×*g* at 4 °C for 20 min. Then the supernatant was separated and stored at − 80 °C, and the antioxidant enzyme activities were measured with commercial kits according to the manufacturer's instructions (Ghasemnejad-berenji et al. 2017).

Estimation of malondialdehyde content

In order to measure malondialdehyde (MDA) levels, 600 μL of supernatant was mixed with 150 μL of TBA (0.67% w/v) and kept in boiling water bath (95 °C) for 30 min. Then thiobarbituric acid reactive substances (TBARS) complex was extracted with n-butyl alcohol by vigorous shaking, cooled at ambient temperature, and centrifuged. The absorbance of TBARS was measured at 532 nm. The calibration curve of tetraethoxypropane standard solutions was used to measure the concentrations of TBARS in testis tissue samples (Asghari et al. 2017; Yazdani et al. 2019).

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay is done on the basis of the antioxidant potential of the sample to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The reagents included 300 mmol/l of acetate buffer (pH 3.6), 10 mmol/l 2,4,6-tri-pyridyl-s-triazine (TPTZ) in 40 mmol/l HCl, and 20 mmol/l $FeCl_3.6H_2O$. Working FRAP reagent was prepared by mixing 50 ml acetate buffer, 5 ml TPTZ solution, and 5 ml FeCl3 solution. A volume of supernatant (10 μL) was freshly added to 300 ml reagent warmed at 37 °C. Interaction between TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) and ferrous iron generates a blue color complex with a maximum absorbance at 593 nm. Ethanolic solutions of known ferrous iron concentration were used to prepare the calibration curve (Benzie and Strain 1996). The calibration curve of ferrous iron standard solutions was used to determine FRAP values in testis tissue samples. Data were expressed as μmol Fe^{3+} reduced to Fe^{2+} per mg protein.

Determination of reduced glutathione

To determine glutathione (GSH) levels (14), a volume of supernatant (10 μL) was mixed with 200 μL of tris-ethylenediaminetetraacetic acid (EDTA) buffer (Tris base (0.25 M), EDTA (20 mM), pH 8.2), and the absorbance at 412 nm was recorded (A_1). Then 4 μL of DTNB (5, 5-dithiobis-2-nitrobenzoic acid) (10 mM) in methanol was added, and the changes in absorbance were recorded again after 15 min (A_2) together with a DTNB blank (B). The content of reduced GSH was calculated as follows:

$$(A_2 - A_1 - B) \times 1.57 \text{ mM}.$$

Sperm parameters analysis

To evaluate sperm parameters, the caudal part of both epididymis from each rat were removed and immediately transferred to a Petri dish containing 10 mL Ham's F10 medium and then cut to several pieces and incubated at 37 °C in 5% CO_2 in order to swim-out spermatozoa into medium. In order to assess sperm motility, 10 μL of sperm sample was placed on a pre-heated Neubauer slide, and then the percentage of motility was determined under light microscope with 400× magnification (Ghasemnejad-Berenji et al. 2018b). For evaluating sperm count, a 1:50 diluted from sperm sample with distilled water was prepared in a micro tube, and then 10 μL of this mixture was placed on a Neubauer slide, and the number of sperm was counted using a light microscope with a magnification of 400× (Agarwal et al. 2008). The percentage of sperm with abnormal morphology for each rat was measured using the smears stained by eosin nigrosine (Narayana et al. 2002).

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed by SPSS 16.0. One-way analysis of variance (ANOVA) was used to determine statistical significance, followed by the post hoc Tukey analysis to establish the statistical difference between the treatment groups. A $p > 0.05$ was considered to be statistically significant.

Results

Coefficient of the testis to body weight

The results of coefficients of the testis to body weight were illustrated in Fig. 1. There were found no significant differences among different treated groups in terms of body weight (Table 1). However, the coefficient of the testis in the TiO_2-treated group was significantly decreased in comparison with the control group ($p < 0.05$). In thymol-treated groups, the coefficient of the testis to body weight were noticeably increased in a dose-dependent manner, and there was no significant difference between the control group and the group treated with TiO_2 plus the high dose of thymol ($p > 0.05$). The epithelial height and diameter of 10 round-shaped seminiferous tubules from each rat were randomly selected and reported. For this purpose, two diameters perpendicular to each other were measured in each seminiferous tubules, and their average was determined. To determine the average of the epithelial height of the same seminiferous tubules, we measured it from the basement membrane to the luminal surface at two locations. The measurements were performed using a light microscope equipped to micrometer lens (Table 2).

Antioxidative enzyme activities in rat testis

The activity of CAT, SOD, and GPx was significantly decreased following oral administration of TiO_2 nanoparticles for 60 consecutive days. Pretreatment with thymol prior to TiO_2 administration improved these enzyme activity. Although the low dose of thymol significantly increased the activity of these antioxidant enzymes, there was significant difference between this group and the control group. However, the activity of antioxidant enzymes in rats treated with the high dose of thymol was restored to control group level ($p < 0.05$, Table 3).

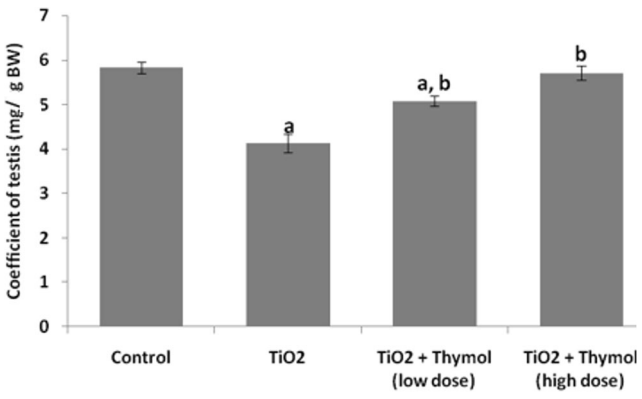

Fig. 1 The organ coefficient was calculated after sub-chronic TiO_2 exposure for consecutive 60 days. *BW*, body weight. Data represent mean ± SEM, $n = 6$ per group. Significantly different from the control $p < 0.05$ (a), significantly different from the TiO_2 group at $p < 0.05$ (b)

Table 1 Effects of thymol on absolute reproductive organ weights in TiO2-treated male rats

Group	Body weight (g)	Weight of right testis (g)
Control	398.71 ± 5.3	2.33 ± 0.04
TiO_2 (100 mg/kg)	381.13 ± 7.04	1.57 ± 0.18[a]
TiO_2 + thymol (low dose)	389.69 ± 2.81	1.98 ± 0.09
TiO_2 + thymol (high dose)	386.71 ± 3.08	2.21 ± 0.11[b]

Data are mean ± SEM of six animals in each group

[a] Significantly different from the control $p < 0.05$

[b] Significantly different from the TiO_2 group at $p < 0.05$

MDA, GSH levels, and FRAP values in rat testis

Oral administration of TiO_2 nanoparticles significantly elevated the MDA levels ($p < 0.05$). The administration of thymol at both doses improved this change in MDA level, and the group treated with TiO_2 plus the high dose of thymol was comparable to the control group (Table 4) ($p > 0.05$). The levels of FRAP and GSH (Table 4) in the TiO_2-treated group significantly reduced in comparison with the control group ($p < 0.05$). Pretreatment with thymol before TiO_2 administration led to significant elevation in the GSH and FRAP levels compared with TiO_2-treated group ($p < 0.05$).

Sperm parameters (count, motility, and abnormal sperm)

The number of sperm and percentage of motility in TiO_2-treated group was significantly reduced in comparison with the control group ($p < 0.05$, Table 5). Moreover, oral administration of TiO_2 nanoparticles for 60 days caused a significant increment in the percentage of sperm with abnormal morphology. As illustrated in Table 3, all alterations in sperm parameters were significantly improved in the groups that orally received thymol before TiO_2 administration.

Histopathological findings

The light microscope findings for testis sections of all groups are illustrated in Fig. 2. Histological examination of the control group showed that the seminiferous tubules (ST) interstitial spaces (IS) are normal and contain sperm and Leydig cells (Fig. 2a). The TiO_2 group showed ST with irregular shape, and lumens contain very little spermatozoa and the interstitial space widening with reduced number of Leydig cells (Fig. 2b). The testicular tissue in the group treated with TiO_2 plus the low dose of thymol (10 mg/kg) showed little recovery and more spermatogonic cells and reduced edema in interstitial space (Fig. 2c). The testicular tissue of rat treated with TiO_2 plus the high dose of thymol (30 mg/kg) showed preserved ST as well as preserved spermatogenesis (Fig. 2d).

Table 2 Histological evaluation of the testis using diameter of seminiferous tubular (ST) diameter values and height of ST epithelium

Group	Height of ST epithelium (μm)	Diameter of ST (μm)
Control	611.9 ± 6.4	936.8 ± 12.4
TiO_2 (100 mg/kg)	492.4 ± 5.9 [a]	713.3 ± 15.2[a]
TiO_2 + thymol (low dose)	547.7 ± 5.8 [a,b]	802.8 ± 24.1[a,b]
TiO_2+ thymol (high dose)	586.8 ± 7.6 [b]	853.7 ± 20.4[a,b]

Data are mean ± SEM of six animals in each group

[a] Significantly different from the control $p < 0.05$

[b] Significantly different from the TiO_2 group at $p < 0.05$

Table 3 Effects of various treatments on CAT, SOD, and GPx enzyme activities in rat testis tissue

Parameters	Control	TiO_2 (100 mg/kg)	TiO_2 + thymol (low dose)	TiO_2 + thymol (high dose)
CAT (U/mg protein)	5.92 ± 0.31	3.01 ± 0.42[a]	4.70 ± 0.17[a,b]	5.63 ± 0.32[b]
GPx (U/mg protein)	2.13 ± 0.08	1.09 ± 0.10[a]	1.54 ± 0.05[a,b]	1.79 ± 0.07[a,b]
SOD (U/mg protein)	70.51 ± 3.28	27.42 ± 2.19[a]	56.33 ± 1.84[a,b]	65.96 ± 3.62[b]

CAT, catalase; *GPx*, glutathione peroxidase; *SOD*, superoxide dismutase

Data are mean ± SEM of six animals in each group

[a] Significantly different from the control $p < 0.05$

[b] Significantly different from the TiO_2 group at $p < 0.05$

Discussion

Many investigations have been done on the harmful effects of TiO_2 nanoparticles on the male reproductive system, but few medications have been introduced to decrease these negative effects (Hong et al. 2015a, b; Narayana et al. 2002; Orazizadeh et al. 2014). The present study demonstrated the protective effects of thymol on TiO_2-induced male germ cell damage and its ability to improve sperm quality after chronic exposure to TiO_2 nanoparticles. Data from our study revealed that the activity of CAT, SOD, and GPx in testis tissue was significantly decreased after chronic exposure to TiO_2 nanoparticles, and pretreatment with thymol ameliorated the activity of all these three antioxidative enzymes. Moreover, the results of the present study showed that the MDA level was significantly increased, and the FRAP and GSH levels were meaningfully decreased which indicated the presence of oxidative stress following prolonged exposure to TiO_2 nanoparticles. Previous investigations have reported that nanoparticles can induce generation of reactive oxygen species (ROS) and reduce levels of GSH in the cells, and our findings are in line with these studies and supports the hypothesis that the nanoparticles induce oxidative stress in cells, which ultimately results in cytotoxicity (Jafari et al. 2018; Karimipour et al. 2018; Park et al. 2008). Furthermore,

Table 4 Effects of various treatments on MDA, GSH levels, and FRAP value in rat testis tissue

Parameters	Control	TiO_2 (100 mg/kg)	TiO_2 + thymol (low dose)	TiO_2 + thymol (high dose)
MDA content (nmol/mg protein)	28.34 ± 1.94	76.19 ± 2.71[a]	50.31 ± 1.67[a,b]	31.55 ± 3.07[b]
GSH levels (μmol /mg protein)	0.193 ± 0.009	0.086 ± 0.012[a]	0.156 ± 0.008[b]	0.181 ± 0.013[b]
FRAP value (μmol /mg protein)	13.94 ± 0.47	6.31 ± 0.38[a]	9.16 ± 0.63[a,b]	10.04 ± 0.71[a,b]

MDA, malondialdehyde; *GSH*, glutathione; *FRAP*, ferric reducing ability of plasma

Data are mean ± SEM of six animals in each group

[a] Significantly different from the control $p < 0.05$

[b] Significantly different from the TiO_2 group at $p < 0.05$

Table 5 Effects of various treatments on sperm parameters in rats

Parameters	Control	TiO_2 (100 mg/kg)	TiO_2 + thymol (low dose)	TiO_2 + thymol (high dose)
Sperm count/mL	127.6 ± 12.5	72.6 ± 11.12[a]	91.6 ± 5.85[a,b]	100.2 ± 5.06[a,b]
Sperm motility (%)	78.5 ± 2.88	55 ± 3.57[a]	62 ± 5.86[a,b]	64.83 ± 3.97[a,b]
Abnormal sperm (%)	27 ± 2.82	46.66 ± 6.53[a]	36.66 ± 3.77[a,b]	32.83 ± 4.87[a,b]

Data are mean ± SEM of six animals in each group

[a] Significantly different from the control $p < 0.05$

[b] Significantly different from the TiO_2 group at $p < 0.05$

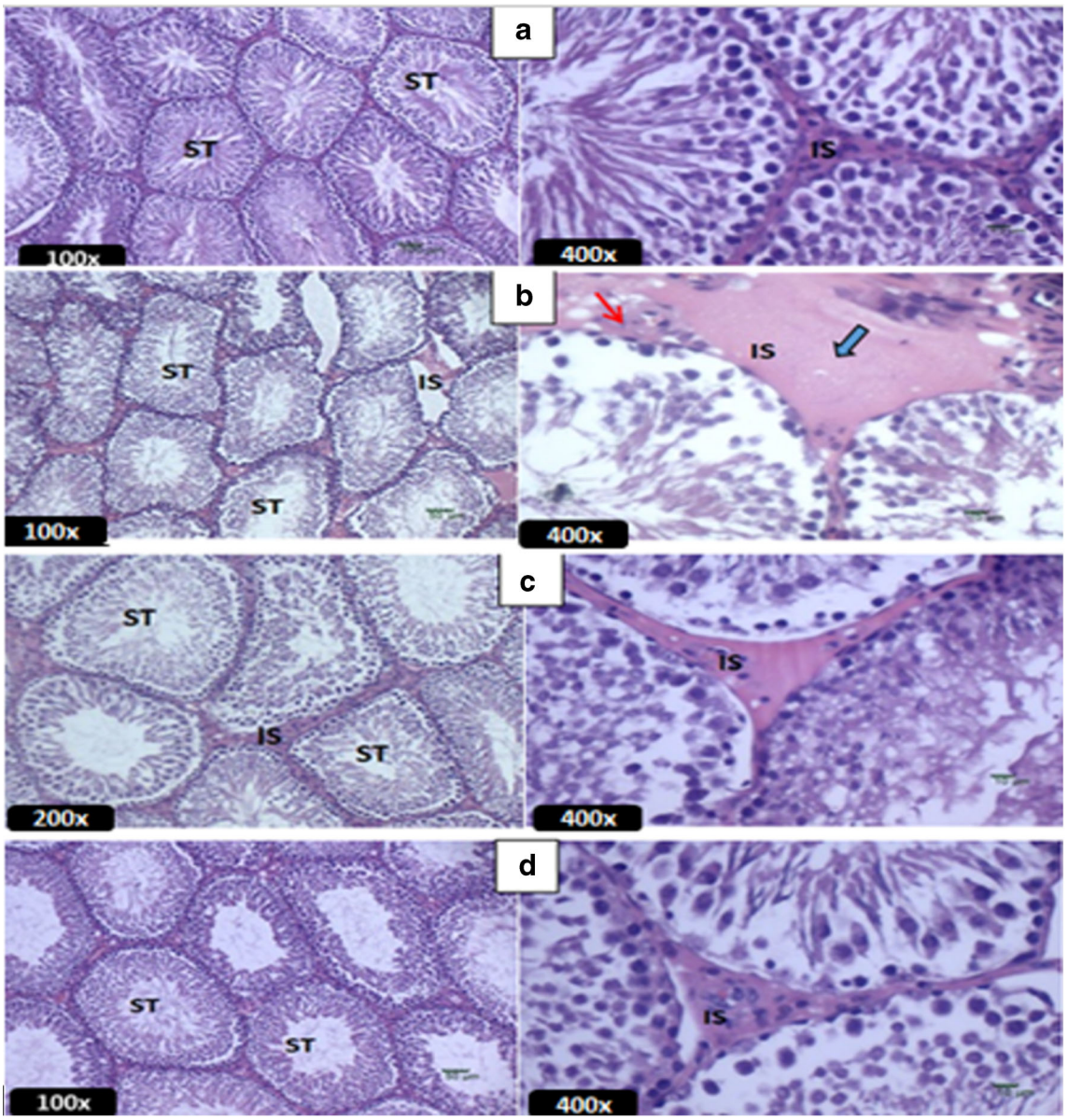

Fig. 2 Development of spermatogenic cells in seminiferous tubules of rat testis. **a** Control group: the histological structure of seminiferous tubules is normal with 5–7 layers of closely and orderly arrayed spermatogenic cells. **b** TiO_2 (100 mg/kg) group,the spermatogenic cells array loosely and disorderly. The gaps between cells were larger than usual (the thick arrow), and some spermatogenic cells desquamated (the thin arrow). **c** TiO_2 + thymol (low dose) group: The spermatogenic cells are less closely arrayed and different stages of spermatogenic cells can be identified. **d** TiO_2 + thymol (high dose) group: The spermatogenic cells in this group are more closely and tightly arrayed than cells in low dose group. Increased cellularity with well-organized tubules and improved tubular structure was observed and the cells array more orderly and closely than other groups except the control group. ST seminiferous tubules, IS interstitial spaces (H&E stain)

according to our results, TiO_2 nanoparticles induced severe histopathological changes such as intraepithelial vacuolization, sloughing, detachment, and seminiferous tubule atrophy and significant decrement in the testis weight. Pre-treatment with thymol ameliorated these histopathological alterations and significantly alleviated the lipid peroxidation as measured by decreased MDA level, increased GSH content, and enhanced activities of CAT, GPX, and SOD in testis tissue. According to our results, both doses of thymol significantly decreased the MDA level; however, the high dose of thymol was more effective, and the MDA level in high-dose pretreated group was near to that of the control group. GSH levels in testis tissue of rats intoxicated with TiO_2 nanoparticles were meaningfully reduced, and the treatment with both doses of thymol led to a significant elevation in the GSH levels in comparison with TiO_2-treated group. The results of the present study are consistent with our previous study in which thymol could effectively protect against TiO_2 nanoparticle–induced hepatotoxicity in male Wistar rats via preventing oxidative stress and ameliorating lipid peroxidation (Jafari et al. 2018).These results could be ascribed to the potential antioxidant activity of thymol. Furthermore, our findings are in agreement with those obtained by El-Sayed et al. (El-Sayed et al. 2016), who demonstrated that thymol prevents cisplatin-induced nephrotoxicity by reducing of oxidative stress, and the study of Meeran and colleagues (El-Sayed et al. 2016; Meeran et al. 2017) who revealed the beneficial role of thymol on lipid peroxidation in isoproterenol-induced myocardial infarction in rats. In another study by Palabiyik et al.(Palabiyik et al. 2016), it was found that thymol could enhance the activity of antioxidant enzymes and also prevent lipid peroxidation induced by acetaminophen. According to our findings, TiO_2 nanoparticle exposure significantly elevated sperm abnormality and decreased sperm density and motility, and pretreatment with thymol effectively attenuated all these alterations. The results showed that sperm abnormality in thymol-pretreated rats was nearly 1.7-fold lesser than TiO_2-intoxicated animals. This amelioration could be due to the protective effects of thymol against TiO_2-induced oxidative stress damages in germ cells. These findings are in line with the study which reported that the oxidative damage induced by hexavalent chromium can be inhibited by the use of thymol in rats (Abd-Elhakim and Mohamed 2016). Beneficial role of thymol against oxidative damage are ascribed to the phenolic hydroxyl group in its structure. It is believed that low doses of phenolic compounds protect cells from the damaging effects of ROS by absorbing and neutralizing them. Also, thymol may potentiate the activity of endogenous antioxidant enzymes (e.g., CAT, GPx, and SOD), and the levels of non-enzymatic antioxidants (e.g., GSH) (Meeran et al. 2017).

Conclusion

In summary, the mechanism of reproductive toxicity of TiO_2 nanoparticles on rat testes consists in the induction of oxidative stress, which may lead to germ cells injury. Treatment of male rats with thymol was shown to be effective in preventing the toxic effects induced by TiO_2 nanoparticles. The probable mechanism of action of thymol might be by decreasing the lipid peroxidation and by increasing the antioxidant power of germ cells.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abd-Elhakim YM, Mohamed WA (2016) Assessment of the role of thymol in combating chromium (VI)-induced oxidative stress in isolated rat erythrocytes in vitro. Toxicol Environ Chem 98:1227–1240

Aboelwafa HR, Yousef HN (2015) The ameliorative effect of thymol against hydrocortisone-induced hepatic oxidative stress injury in adult male rats. Biochem Cell Biol 93:282–289

Agarwal A, Makker K, Sharma R (2008) Clinical relevance of oxidative stress in male factor infertility: an update. Am J Reprod Immunol 59: 2–11

Alaee S, Ilani M (2017) Effect of titanium dioxide nanoparticles on male and female reproductive systems. J Adv Med Sci Appl Technol 3:3–8

Asghari MH, Moloudizargari M, Baeeri M, Baghaei A, Rahimifard M, Solgi R, Jafari A, Aminjan HH, Hassani S, Moghadamnia AA (2017) On the mechanisms of melatonin in protection of aluminum phosphide cardiotoxicity. Arch Toxicol 91:3109–3120

Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70–76

Cardoso ES, Santana TA, Diniz PBF, Montalvão MM, Bani CC, Thomazzi SM (2016) Thymol accelerates the recovery of the skeletal muscle of mice injured with cardiotoxin. J Pharm Pharmacol 68: 352–360

Chen E, Ruvalcaba M, Araujo L, Chapman R, Chin W-C (2008) Ultrafine titanium dioxide nanoparticles induce cell death in human bronchial epithelial cells. J Exp Nanosci 3:171–183

El-Sayed ESM, Mansour AM, Abdul-Hameed MS (2016) Thymol and carvacrol prevent doxorubicin-induced cardiotoxicity by abrogation of oxidative stress, inflammation, and apoptosis in rats. J Biochem Mol Toxicol 30:37–44

Ghaffarian-Bahraman A, Shahroozian I, Jafari A, Ghazi-Khansari M (2014) Protective effect of magnesium and selenium on cadmium toxicity in the isolated perfused rat liver system. Acta Med Iran 52: 872–878

Ghasemnejad-berenji M, Ghazi-Khansari M, Yazdani I, Saravi SSS, Nobakht M, Abdollahi A, Ansari JM, Ghasemnejad-berenji H, Pashapour S, Dehpour AR (2017) Rapamycin protects testes against germ cell apoptosis and oxidative stress induced by testicular ischemia-reperfusion. Iran J Basic Med Sci 20:905

Ghasemnejad-berenji M, Ghazi-Khansari M, Pashapour S, Jafari A, Yazdani I, Ghasemnejad-berenji H, Saravi SSS, Sadeghpour S, Nobakht M, Abdollahi A (2018a) Synergistic effect of rapamycin and metformin against germ cell apoptosis and oxidative stress after testicular torsion/detorsion-induced ischemia/reperfusion in rats. Biomed Pharmacother 105:645–651

Ghasemnejad-Berenji M, Ghazi-Khansari M, Yazdani I, Nobakht M, Abdollahi A, Ghasemnejad-Berenji H, Mohajer Ansari J, Pashapour S, Dehpour A (2018b) Effect of metformin on germ cell-specific apoptosis, oxidative stress and epididymal sperm quality after testicular torsion/detorsion in rats. Andrologia 50:e12846

Hong F, Si W, Zhao X, Wang L, Zhou Y, Chen M, Ge Y, Zhang Q, Wang Y, Zhang J (2015a) TiO2 nanoparticle exposure decreases spermatogenesis via biochemical dysfunctions in the testis of male mice. J Agric Food Chem 63:7084–7092

Hong F, Zhao X, Si W, Ze Y, Wang L, Zhou Y, Hong J, Yu X, Sheng L, Liu D (2015b) Decreased spermatogenesis led to alterations of testis-specific gene expression in male mice following nano-TiO2 exposure. J Hazard Mater 300:718–728

Iavicoli I, Leso V, Fontana L, Bergamaschi A (2011) Toxicological effects of titanium dioxide nanoparticles: a review of in vitro mammalian studies. Eur Rev Med Pharmacol Sci 15:481–508

Jafari A, Rasmi Y, Hajaghazadeh M, Karimipour M (2018) Hepatoprotective effect of thymol against subchronic toxicity of titanium dioxide nanoparticles: biochemical and histological evidences. Environ Toxicol Pharmacol 58:29–36

Karimipour M, Javanmard MZ, Ahmadi A, Jafari A (2018) Oral administration of titanium dioxide nanoparticle through ovarian tissue alterations impairs mice embryonic development. Int J Reprod Biomed 16:397

Meeran N, Fizur M, Javed H, Al Taee H, Azimullah S, Ojha SK (2017) Pharmacological properties and molecular mechanisms of thymol: prospects for its therapeutic potential and pharmaceutical development. Front Pharmacol 8:380

Narayana K, D'Souza UJ, Rao KS (2002) Ribavirin-induced sperm shape abnormalities in Wistar rat. Mutat Res/Genet Toxicol Enviro Mutagen 513:193–196

Orazizadeh M, Khorsandi L, Absalan F, Hashemitabar M, Daneshi E (2014) Effect of beta-carotene on titanium oxide nanoparticles-induced testicular toxicity in mice. J Assist Reprod Genet 31:561–568

Palabiyik S, Karakus E, Halici Z, Cadirci E, Bayir Y, Ayaz G, Cinar I (2016) The protective effects of carvacrol and thymol against paracetamol–induced toxicity on human hepatocellular carcinoma cell lines (HepG2). Hum Exp Toxicol 35:1252–1263

Park E-J, Yi J, Chung K-H, Ryu D-Y, Choi J, Park K (2008) Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells. Toxicol Lett 180:222–229

Shakeel M, Jabeen F, Shabbir S, Asghar MS, Khan MS, Chaudhry AS (2016) Toxicity of nano-titanium dioxide (TiO 2-NP) through various routes of exposure: a review. Biol Trace Elem Res 172:1–36

Weir A, Westerhoff P, Fabricius L, Hristovski K, Von Goetz N (2012) Titanium dioxide nanoparticles in food and personal care products. Environ Sci Technol 46:2242–2250

Yazdani I, Majdan R, Ghasemnejad-berenji M, Dehpour AR (2019) Comparison of multiple doses of cyclosporine a on germ cell apoptosis and epididymal sperm parameters after testicular ischemia/reperfusion in rats. Exp Mol Pathol 104271