Biological Trace Element Research
https://doi.org/10.1007/s12011-019-01836-x

The Effect of Cerium Oxide During Pregnancy on the Development of the Testicular Tissue of Newborn NMRI Mice

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Received: 2 March 2019 / Accepted: 15 July 2019

Abstract

Cerium(IV) oxide is widely used as a catalyst in all aspects of human life and human beings are exposed to these materials. The purpose of this experimental study was to investigate the effect of CeO_2 during pregnancy on alterations in the testis tissue and blood biochemical parameters in newborn mice. Pregnant NMRI mice were divided randomly into five groups ($n = 6$ for each group) including one control group and 4 treatment groups. Injection of CeO_2 solution was administered intraperitoneally at the doses of 10, 25, 80, and 250 mg/kg.bw, respectively, on GD 7 and GD 14. At the end of treatment period, the testicular histological and biochemical parameters of 2- and 6-day-old newborns were analyzed, as well as the biochemical parameters in serum samples of 15-day-old newborns. The number of spermatogonia, Sertoli, and Leydig cells in the testis of the 2-day-old newborn and spermatogonia and Leydig cells in the testis of the 6-day-old newborns in the 250 mg/kg.bw CeO_2 treatment group was significantly reduced compared with the control group ($P < 0.05$). Testis MDA of the 2- and 6-day-old newborns in the treated group receiving 250 mg/kg.bw of CeO_2 was significantly higher than the control group ($P < 0.001$). There was no significant difference between serum MDA and TAC levels between the treated groups with different doses of CeO_2 compared with the control group. Therefore, CeO_2 given to dams during pregnancy may affect the testicular tissue and blood biochemical parameters in neonates and may be dose-dependent.

Keywords Cerium oxide · Pregnant mice · Newborn · Testicular tissue

Abbreviations

bw	Body weight
Ce	Cerium
CeO_2	Cerium(IV) oxide
dd	Double distilled
dpp	Days post-partum
D2	2-day-old infant
D6	6-day-old infant
°C	Degree centigrade
Fig	Figure
g	Gram
GD	Gestational day
HCL	Hydrochloric acid
h	Hour
i.p	Intraperitoneal
L	Leydig cells
μl	Microliter
MDA	Malondialdehyde concentration
μm	Micrometer
min	Minute
mm^3	Cubic millimeters
mg/kg.bw	Milligrams per kilogram of body weight
ml	Milliliter
nm	Nanometer
nmol	Nanomolar
KCL	Potassium chloride
REEs	Rare earth elements
St	Sertoli cells
Sp	Spermatogonia cells
TAC	Total antioxidant capacity
TCA	Tricloric acid
TPTZ	2,4,6Tripyridyl-s-triazine
TBA	Thiobarbituric acid
V	Volume
W	Weight

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Published online: 14 August 2019

Introduction

Rare earth elements (REEs) due to their unique physical and chemical properties are widely used in many fields such as medicine, agriculture, industry, and food [1, 2].

Cerium (Ce) is a member of the lanthanide sequences and is the most abundant element of the rare earth elements in the earth's crust. It can exist in both the + 3 (Ce^{3+} = [Xe] 4f12) and + 4 (Ce = [Xe]) oxidation states and has high electrical conductivity and softness and it also is a very reactive and powerful oxidizing factor that is stabilized when associated with an oxygen ligand. The highest exposure to cerium compounds is through exposure to cerium oxide (CeO_2) [3–7].

Cerium has a variety of applications in many fields for example widespread use as catalysts, oxygen sensors, solar cells, solid fuel cells, UV blocking agent and polishing agents, ultraviolet absorbents, as phosphor/luminescence, automotive catalytic converters, gas sensors, oxygen pumps, and metallurgical and glass/ceramic [2, 8–10].

In recent years, attention has been paid to the antioxidant properties of cerium and its application in the fields of medicine and biotechnology. Research on the antioxidant effects of cerium oxide has increased in the treatment of diseases, for example, age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa, aging, trauma, Alzheimer, and Parkinson [4, 11, 12].

Due to the ever-increasing use of nano- and microparticles of cerium oxide in all aspects of human life and human exposure to these materials, research into the effects of these substances on human health is essential. According to studies that have been done so far, the effects of cerium oxide on human and animal cells have been reported to be contradictory.

A number of articles have been published showing that exposure to cerium oxide nanoparticles can have adverse effects on human health through the production of active oxygen species leading to oxidative stress, inflammation, and cell death–induced stress (apoptosis) [8, 13]. A study has shown cerium oxide nanoparticles are able to induce angiogenesis in both vivo and in vitro environments where abnormal angiogenesis can cause diseases such as cancer, chronic inflammation, diabetic retinopathy, and arthritis [14].

In contrast, some studies have reported that cerium oxide nanoparticles exhibit antioxidant properties that promote cell survival under conditions of oxidative stress and a potential for therapeutic and cardiac, neurological, and ocular protection purposes [12, 15]; another study has also shown that the optimal concentration of cerium oxide nanoparticles increases the activity of cell division of the initial embryonic fibroblasts in vitro [16].

Also the previous studies have shown that cerium oxide is able to cross the placental barrier and influences the tissues of the fetus [2]. There is little information about the potential effects of CeO_2 in the reproductive system and gametes. In a study, into the effects of CeO_2 on the testis of rats showed that it increases the quality parameters of sperm [17], while a recent study that has investigated the effects of cerium oxide on fertilization in mice has reported the genetic toxicity of malformed gametes and harmful effects on mice fertilization [18]. There is no report on the role of cerium on testicular tissues of neonatal mice, and due to the fact that the adult testicular tissue function depends on the development of the testicular tissue during fetal and neonatal period, we have focused on the effect of cerium oxide on the testis of the newborn mice during pregnancy.

Materials and Methods

Materials

The cerium(IV) oxide (CeO_2) powder with a diameter of < 5 μm, assay 99.9% trace metals basis, and density of 7.13 g/ml at 25°c(lit.) tested in this study was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA).

Different concentrations of the CeO_2 stock suspensions were prepared freshly in double distilled (dd) water. Ultrasonic vibration (100 W, 30 kHz) was performed for 15 min before use.

Animal

Forty-five adult NMRI mice (15 male and 30 female) weighing 25–30 g were purchased from the Pasteur Institute of Iran and were acclimatized for 1 week before the start of the experiment. Male and female mice were kept in separate cages and were maintained at a temperature of 22 ± 2 °C with 12:12-h light/dark cycle, with 55 ± 5% humidity, and ventilation of 10–20 times/h. Feed and water were provided ad libitum. After 1 week of acclimatization, mice were bred naturally, and a single male and two female mice were housed together for 48 h. Female mice were checked for vaginal plugs each morning and evening. When a vaginal plug was observed, the female mouse was separated from the male mouse and observation of a vaginal plug designated gestation day (GD) 0. Pregnant mice were divided randomly into five groups ($n = 6$ for each group): control group was treated with 100 μl of distilled (dd) water and 4 treatment groups received 100 μl of CeO_2 respectively prepared with selected doses (10, 25, 80, 250 mg/kg.bw) in distilled (dd) water, and the administration of intraperitoneal (i.p) injection was performed on GD 7 and GD 14.

At the end of the pregnancy, 2- and 6-day-old male newborns were used in this study for histological evaluation. Also, 15-day-old male newborns were used for serum preparation.

The study was approved by the Institutional Animal Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran. Ethical clearance number for this study is IR.KUMS.REC.1397.1004.

Histological Evaluation of the Testis

Newborn male mice aged 2 and 6 days post-partum (dpp) were first weighed and then were killed. The right testis were excised and rinsed in distilled water, then were weighed and fixed for 48 h in a fixative solution MDF (modified Davidson's fluid) and were embedded in paraffin for histological examination. Ten sections (4 μm) from each testis were cut at intervals of 20 μm and were stained with Heidenhain azan [19, 20]. A study on testicular tissue was done using a light microscope (Leica Microsystem (Switzerland) Limited). Histological evaluations were performed using images at × 1000 magnification. A total of six samples were obtained from six neonates in each 2-day-, 6-day-, and 15-day-old neonates treatment group.

Two slides per sample were scored. The number of spermatogonia, Sertoli, and Leydig cells was counted using a special probe for counting in the average number of 30 globular seminiferous tubules of each sample [20–22].

Volumes of the testis were assessed by the Cavalieri method [23]. Thus, from all 4-μm sections, randomly systematic and with an identical distance, an average of 12 sections was selected and each image was viewed at a magnification of 400 by an optical microscope. The pre-designed point probe is randomly uniform and without any bias on the image of each of the sections and the points encountered with the whole testicle image were counted.

The total volume of the testis was counted in all the slices using the following formula and by entering the sum of the total points ($\sum_{i=1}^{n} P$). Thickness between selected sections (t) and the level of the point probe ($a(p)$) were estimated.

$$V_{(\text{total})} = \sum_{i=1}^{n} P \times a(p) \times t$$

Biochemical Studies

In order to determine the serum biochemical parameters, blood samples were taken from the heart of 15-day-old neonates, and blood samples were centrifuged for 10 min at a rate of 16,000g for plasma separation from the serum. Then, the serum was centrifuged once more for 5 min at the same speed so that the serum became completely free of red blood cells. Serum was stored at − 80 °C.

Evaluation of Serum Lipid Peroxidation Level by Measuring the Malondialdehyde Concentration

In order to measure malondialdehyde, Buege and Aust methods were used. In this method, the *malondialdehyde concentration (MDA)* reacts with *thiobarbituric acid (TBA)* and produces a pink compound that can absorb rays with a wavelength of about 535–532 nm.

MDA measurement method: At first, *TCA-TBA-HCL* solution containing Trichloroacetic acid (TCA) 15% g/ml, TBA 0.375% (g/ml), and hydrochloric acid (HCL) 25% normal was prepared. Then 0.5 ml of the serum was mixed with 1 ml of TCA-TBA-HCL solution and the samples were placed in boiling bain-marie for 15 min. Then, the samples were cooled by cold water and were centrifuged for 10 min. The liquid was dissolved and absorbed at 532 nm against a blank containing all compounds except the sample. The concentration of MDA was calculated using its extinction coefficient, which was equal to 1.56×10^5 M^{-1} cm^{-1} expressed in nmol/ml [24, 25].

Total Antioxidant Capacity

The *FRAP* method was used to determine the total antioxidant potential period. 0.5 ml of serum sample was incubated with 1.5 ml of the reaction mixture containing 2,4,6-tripyridyl-s-triazine (TPTZ), $FeCl_3$ and sodium acetate buffer for 10 min at 37 °C and its absorbance was read at 593 nm using a spectrophotometer. The serum level of total antioxidant capacity (TAC) was calculated using an absorbance standard curve. This method is based on the ability of the serum to recover Fe^{3+} (ferric) ions into Fe^{2+} (ferro) in the presence of TPTZ substance. The *FRAP* measurement is considered as a direct test to evaluate total antioxidant power [26].

Evaluation of Lipid Peroxidation of the Tissue by Measuring Malondialdehyde

In order to determine the MDA of the tissue, the left testis of the 2- and 6-day newborns was placed in a 1:10 KCL solution and homogenized for 2 min then mixed with a solution of TCA-TBA-HCL. The new solution was put in a bain-marie for 15 min, and after cooling, the microtube was centrifuged for about 10 min at a rate of 1000g. After centrifuging, the microtube was dissolved and the absorbance was measured at 532 nm. Results were expressed in nanomole to the gram of tissue (nmol/g) [15].

Statistical Analysis

Data were analyzed by SPSS software version 16 using one-way ANOVA and Tukey's tests. The mean difference was considered significant at $P < 0.05$ level.

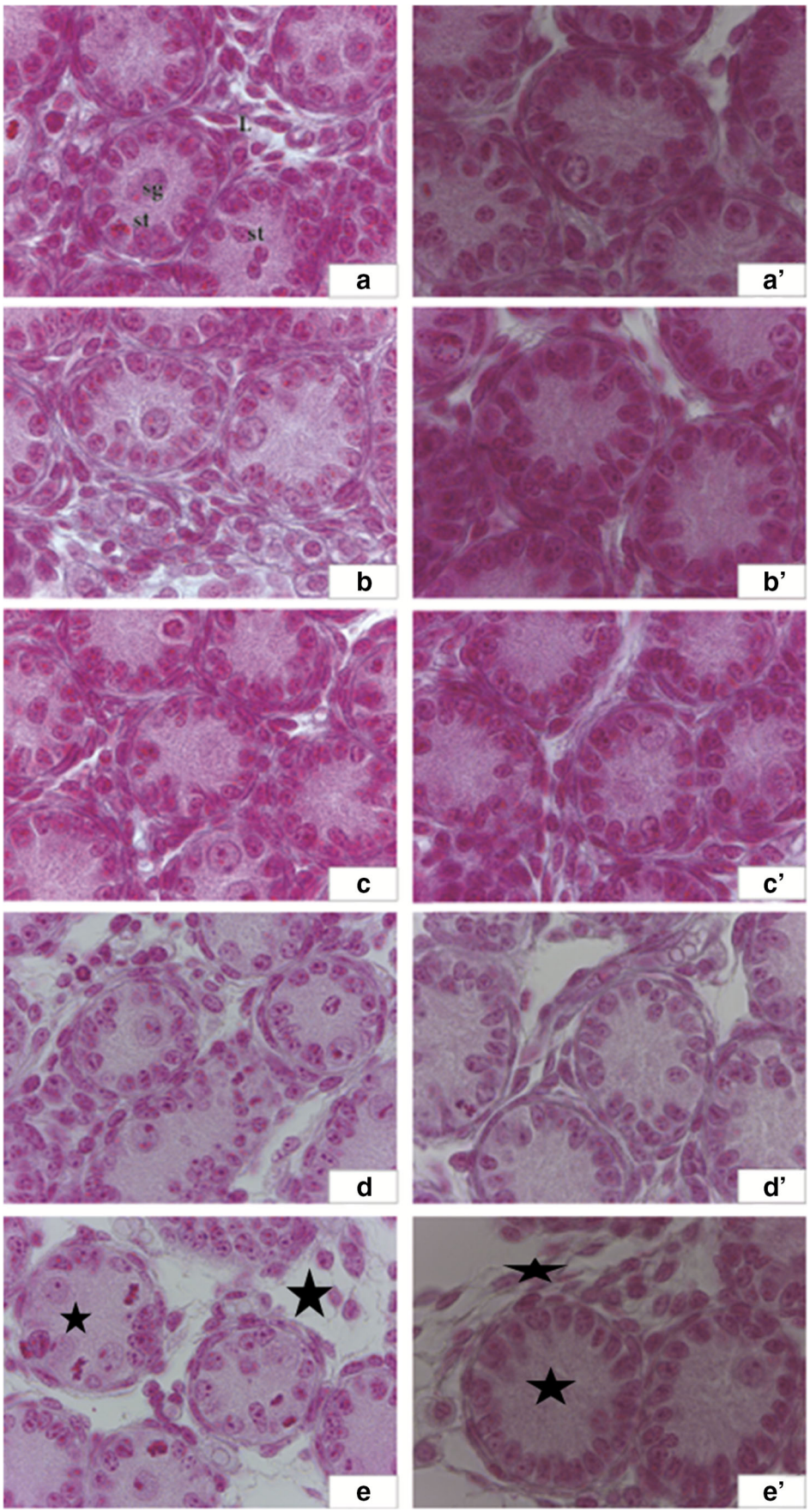

Fig. 1 Microscopic images of testicular tissue from the 2- and 6-day-old mice newborns (A–E, 2-day-old newborn testis; A′–E′, 6-day-old newborn testis). Four-micron sections (stained with Heidenhain azan, magnification × 1000) show the histopathological changes in the testis. AA′, control; BB′, CeO_2 (10 mg/kg.bw); CC′, CeO_2 (25 mg/kg.bw); DD′, CeO_2 (80 mg/kg.bw); EE′, CeO_2 (250 mg/kg.bw). Sertoli cells (St, small ovoid nuclei, arranged perpendicular to the basement membrane) and type A spermatogonia (Sg, large spherical nuclei) is made up of the seminiferous epithelium. Sertoli cell cytoplasm fills the future lumen of the seminiferous tubules; L, Leydig cells are located among the lumens, smaller than the rest of the cells. (B–D) (B′–D′) Histological changes are not significant compared with the control group. (E) The number of spermatogonia, Sertoli, and Leydig cells in the 2-day-old newborn was decreased and the lumen space is more hollow (star). (E′) The number of spermatogonia and Leydig cells in the 6-day-old newborn was decreased and the lumen space is more hollow (star)

Table 1 Comparison of the number of spermatogonia (Sp), Sertoli cells (St), and Leydig cells (L) of testicular tissue of a 2-day-old newborn (D2) and a 6-day-old newborn (D6) in treated groups with different doses of cerium oxide with control group. Data are presented as mean ± SD. Means with different letter codes have significant difference with each other (one-way ANOVA, Tukey's test, $P < 0.05$)

Group	Sp (D2)	Sp (D6)	St (D2)	St (D6)	L (D6)	L (D2)
Control	202.17 ± 16.89^{a}	309 ± 37.42^{ab}	148.33 ± 5.57^{a}	197 ± 40.47^{ab}	81.67 ± 17.68^{a}	76.17 ± 10.75^{a}
CeO_2 (10 mg/kg.bw)	210.33 ± 17.38^{a}	330.17 ± 37.36^{a}	155 ± 5.32^{a}	217.5 ± 36.01^{a}	88.83 ± 8.03^{a}	79.67 ± 6.34^{a}
CeO_2 (25 mg/kg.bw)	185.65 ± 9.26^{ab}	302.33 ± 51.85^{ab}	156.5 ± 5.12^{a}	180.17 ± 18.37^{ab}	78.33 ± 5.09^{ab}	70.67 ± 10.55^{a}
CeO_2 (80 mg/kg.bw)	183.33 ± 14.02^{ab}	262.67 ± 12.56^{bc}	141 ± 20.05^{ab}	167.83 ± 16.89^{b}	76.67 ± 10.8^{ab}	66.33 ± 5.82^{ab}
CeO_2 (250 mg/kg.bw)	157.33 ± 24.55^{b}	226 ± 16.13^{c}	125.33 ± 5.85^{b}	160.33 ± 14.26^{b}	60.67 ± 3.88^{b}	56.17 ± 3.37^{b}

Results

Histological Evaluation of the Testis

Evaluation and counting of newborn testicular tissue cells were performed using a light microscope (Fig. 1).

Histological Evaluation of Testis of the 2-Day-Old Newborn

The number of spermatogonia in the testis of the 2-day-old newborn was decreased significantly in the treated group with a dose of 250 mg/kg.bw of cerium oxide compared with control)$P < 0.001$(. The number of spermatogonia was increased slightly in the treated group with a dose of 10 mg/kg.bw of CeO_2 compared with the control group, but it was not statistically significant. In other groups, there was no significant change compared with the control.

The number of Sertoli cells in a 2-day-old newborn has a significant decrease in the treated group with a dose of 250 mg/kg.bw of CeO_2 compared with the control group ($P < 0.005$). In other groups, there is no significant change in Sertoli cell count compared with control.

The number of Leydig cells in the 2-day-old newborn was reduced significantly in the treated group with a dose of 250 mg/kg.bw of CeO_2 compared with the control group ($P < 0.002$). In other groups, there is no significant difference compared with the control group (Table 1).

Histological Evaluation of Testis of the 6-Day-Old Newborn

The number of spermatogonia in the testis of the 6-day-old newborn was reduced significantly in the treated group with a dose of 250 mg/kg.bw of CeO_2 compared with control ($P < 0.03$). In other groups, there is no significant change compared with control.

There was no significant difference in Sertoli cell numbers in the 6-day-old newborn between treated groups with different doses and control group.

The number of Leydig cells in the treated group with a dose of 250 mg/kg.bw of CeO_2 was significantly lower than the control group ($P < 0.013$) (Table 1).

Biochemical Evaluations

MDA Level There was no significant difference between serum MDA of the 15-day-old newborn in the control group and other treated groups (Fig. 2). Testis MDA of the 2- and 6-day-old newborns in the treated group with a dose of 250 mg/kg.bw of CeO_2 was significantly higher than control group ($P < 0.001$) (Fig. 3).

TAC Level There was no significant difference between serum of total antioxidant capacity in the treated groups with different doses of CeO_2 compared with the control group (Fig. 4).

Body Weights

Weight of newborn mice in treated groups did not show any significant difference compared with the control group (Table 2).

Weight and Volume of the Testicles

The testicular weight of the 2- and 6-day-old newborns in all treatment groups with different doses of cerium oxide (per

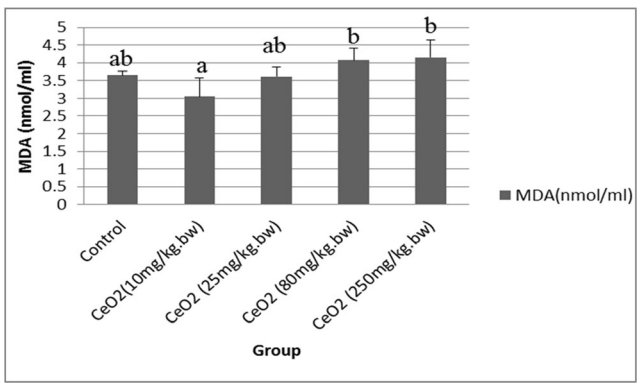

Fig. 2 Comparison of serum malondialdehyde (MDA) in nmol/ml of the 15-day-old newborn mice in treated groups with different doses of cerium oxide (CeO_2) with control group. Data are presented as mean ± SD. Means with different letter codes have significant difference with each other (one-way ANOVA, Tukey's test, $P < 0.05$)

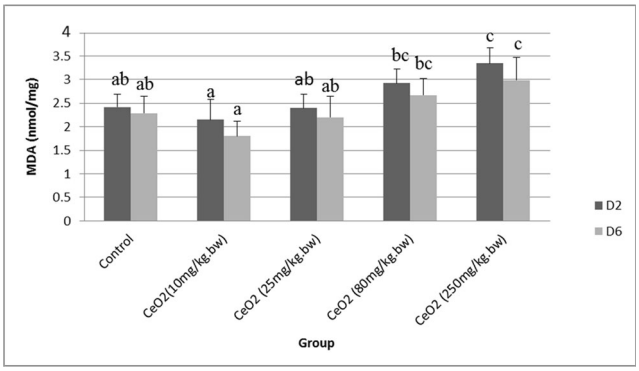

Fig. 3 Comparison of tissue malondialdehyde ((MDA) in nmol/mg) of the 2- and 6-day-old newborn mice (D2, D6) in treated groups with different doses of cerium oxide (CeO_2) with control. Data are presented as mean ± SD. Means with different letter codes have significant difference with each other (one-way ANOVA, Tukey's test, $P < 0.05$)

mg/kg body weight) did not show any significant difference compared with the control group (Table 2).

The 2-day newborn's testicular volume was reduced significantly in the treated group with a dose of 250 mg/kg.bw of CeO_2 compared with the control group ($P < 0.025$). In other groups, there is no significant difference compared with the control group. The 6-day-newborn's testicular volume was not significantly different between the treated groups with different doses of CeO_2 compared with the control group (Table 2).

Discussion

We injected *cerium(IV) oxide* powder with different doses to pregnant mice intraperitoneally according to previous studies. Then, the changes in the testis of newborn mice were evaluated by light microscopy. So far, no study has been done in this regard, and few studies have been done on the potential effects of cerium(IV) oxide on the male reproductive system

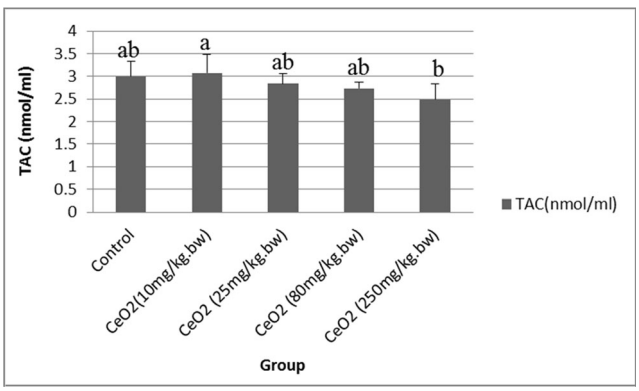

Fig. 4 Comparison of serum total antioxidant capacity (TAC) in nmol/ml of the 15-day-old newborn mice in treated groups with different doses of cerium oxide (CeO_2) with control group. Data are presented as mean ± SD. Means with different letter codes have significant difference with each other (one-way ANOVA, Tukey's test, $P < 0.05$)

and gametes. Studies on the effects of cerium oxide on the reproductive system show conflicting results [1, 5, 27, 28].

One study examined the in vitro effects of rare earth elements (REEs) concentrations in human semen and its relationship with sperm quality; the effect of cerium was also investigated. The results of this study showed that there were both positive and negative correlations between the cerium with semen quality parameters such as volume of ejaculation, sperm concentration, motility, and morphology. It was also shown that increasing the concentration of REEs and cerium has a direct relationship with their negative effect on the quality of sperm and semen [28].

In a study on the effects of the two elements, cerium and lanthanum on the fetus of the sea urchin and sperm, impairment of sperm development, and successful fertilization in the offspring of Ce(IV)-exposed were observed [27], while a study on the testis of rats has shown that nanoparticles of cerium at a dose of 1 mg.kg.bw increased the quality of sperm parameters and the numbers of Leydig cells [17]. An in vitro study was conducted on the effects of CeO_2 nanoparticles concentration (0.01 to 10 mg l^{-1}) on human spermatozoa. This study showed CeO_2 nanoparticles can induce DNA damage in human spermatozoa [29].

Testicular tissue studies of the 2-day-old newborn showed that the number of spermatogonia, Sertoli, and Leydig cells was decreased significantly only in the group treated with a high dose (250 mg/kg.bw) of cerium(IV) oxide in this study compared with the control group ($P < 0.05$). There was no significant difference in the number of testicular tissue cells in the treated groups with doses of 80 mg/kg.bw and 25 mg/kg.bw of CeO_2 compared with the control group. In the treated group with a dose of 10 mg/kg.bw of CeO_2, the increase in the number of spermatogonia, Sertoli, and Leydig cells was observed compared with the control group, but this improvement was not significant compared with the control group (Table 1).

Testicular tissue study of the 6-day-old newborn showed that the number of spermatogonia and Leydig cells in the 6-day-old newborn was reduced significantly only in the treated groups with a dose of 250 mg/kg.bw of CeO_2 compared with control ($P < 0.05$). However, there was no significant difference in the number of Sertoli cells in other treated groups with different doses compared with the control group (Table 1).

The results of this study indicate that the administration of cerium oxide only at high doses causes a significant negative change in testicular tissue compared with the control group. There is no statistically significant change in the administration of cerium oxide at low doses in testicular tissue (Table 1).

In mice, the gametes on the fetal day 6.5 are characterized by BMP signals. Like other body tissues of multicellular organisms, testis development in embryonic period is influenced by the secretion of signals and factors such as hormones, growth factors, and environmental conditions [30].

Table 2 Comparison of mean body weight and testicular (g) and testicular volume (mm^3) of the 2- and 6-day-old newborn mice in different groups: bw (D_2), 2-day-old newborn weight; bw (D_6), 6-day-old newborn weight. Testis W (D_2), 2-day-old newborn testicle weight; testis W (D_6), 6-day-old newborn testicle weight; testis V (D_2), 2-day-old newborn testicle volume; testis V (D_6), 6-day-old newborn testicle volume. Data are presented as mean ± SD. Means with different letter codes have significant difference with each other (one-way ANOVA, Tukey's test, $P < 0.05$)

Group	bw (D_2) (g)	bw (D_6) (g)	Testis W (D_2) (g)	Testis W (D_6) (g)	Testis V (D_2) (mm^3)	Testis V (D_6) (mm^3)
Control	1.97 ± 0.18^{a}	4.09 ± 0.32^{a}	0.0032 ± 0.0009^{ab}	0.0086 ± 0.0018^{ab}	1.18 ± 0.16^{ab}	2.29 ± 0.17^{ab}
CeO_2 (10 mg/kg.bw)	1.98 ± 0.26^{a}	4.15 ± 0.43^{a}	0.0037 ± 0.0005^{a}	0.0093 ± 0.001^{a}	1.27 ± 0.53^{a}	$2.31 \pm .014^{a}$
CeO_2 (25 mg/kg.bw)	1.91 ± 0.72^{a}	3.92 ± 0.44^{a}	0.0033 ± 0.0004^{ab}	0.0086 ± 0.0011^{ab}	1.12 ± 0.89^{ab}	2.17 ± 0.15^{ab}
CeO_2 (80 mg/kg.bw)	1.80 ± 0.16^{a}	3.84 ± 0.19^{a}	0.0030 ± 0.0005^{ab}	0.0069 ± 0.0011^{b}	0.99 ± 0.73^{bc}	2.08 ± 0.11^{ab}
CeO_2 (250 mg/kg.bw)	1.78 ± 0.25^{a}	3.82 ± 0.29^{a}	0.0025 ± 0.0004^{b}	0.0068 ± 0.001^{b}	0.93 ± 0.14^{c}	2.05 ± 0.12^{b}

According to the previous studies, cerium oxide is able to cross the dam's placental barrier [2]. It has also been shown that cerium oxide is able to cross the cell surface and its presence within the cells is limited to particles or macromolecules that are swallowed or phagocytected by cells [31]. Micro- and nanoscale particles are entered into various parts of the body such as the skin, food, medicine, respiration, and breast milk, then entering into the bloodstream, and they are able to cross the placental barrier and the blood-testis barrier and ultimately influencing the testis [2, 32].

Nanoparticle and microparticle toxicity results in excessive release of free radicals and oxidative stress leading to lipid peroxidation, protein oxidation, and DNA damage, which in turn causes inflammatory responses and ultimately apoptosis and can influence the reproductive system [31, 32].

Previous studies have shown that oxidative stress and DNA or protein damage lead to the loss of Leydig cells [17]. Reduction of Leydig cells in the treated group with a dose of 250 mg/kg.bw of cerium oxide compared with the control group could be due to the oxidative stress that is caused by high-dose administration of cerium oxide. Also, reduction of Sertoli cells and spermatogonia can be caused by various reasons including oxidative stress and increased lipid peroxidation. Sex cells due to the high content of unsaturated fatty acids in their membranes are very sensitive to oxidative stress [33]. Reduced antioxidant capacity and spermatogenic cell apoptosis (caused by increasing the expression of pre-apoptotic protein called BAX) are factors that reduce the number of sex cells [34].

Lipids are one of the most important molecules invaded by free radicals. This process leads to lipid peroxidation, which ultimately leads to loss of life and cell death. Cell membranes are rich in unsaturated fatty acids (PUFA—poly unsaturated fatty acid) and therefore are more sensitive to peroxidation than other parts of the cell. Lipid peroxidation leads to a decrease in fluidity of the membrane and the destruction of the building and its operation, and due to oxidation of lipids, the ultimate product is malondialdehyde (MDA) [35, 36]. The presence of MDA is an indicator of free radical damage.

In this experiment, serum MDA of the 15-day-old newborn mice was measured. Results showed that there is no significant difference between the treated groups with different doses of cerium oxide and control group (Fig. 2).

Testis MDA evaluation of the 2- and 6-day-old newborn showed a slight decline in the treated groups with different doses of CeO_2 at a dose of 10 mg/kg.bw of CeO_2 ($P > 0.05$), and also showed that the tissue MDA was increased significantly in the group treated with a dose of 250 mg/kg.bw of CeO_2 ($P < 0.001$); there was no significant difference in other groups compared with the control group (Fig. 3).

There is no significant change between total serum antioxidant capacity in treated groups with different doses of cerium(IV) oxide compared with the control group (Fig. 4). Oxidative stress may occur due to the excessive ROS production or decreased antioxidant levels of the cell [31]. According to the studies conducted so far, some of the cells may have a greater sensitivity to the effects of CeO_2 [37], and tissue environmental conditions such as pH play an important role in determining the oxidation behavior of oxidation cerium oxide so that in normal pH conditions, cerium oxide acts as a cell-dependent antioxidant [17, 38].

The ability of CeO_2 to react with ROS, especially hydrogen peroxide, and superoxide is comparable with biological antioxidants such as superoxide dismutase and catalase. A higher ratio of Ce^{3+}/Ce^{4+} reduces superoxide ions and the antioxidant effect of cerium depends on this ability to convert between two forms. The toxicity of CeO_2 seems to depend on the type of cell and tissue and dose-dependent and the duration of exposure [39].

Determination of body weight and tissue is an important parameter for evaluating the effects and toxicity. In this study, body weight of the newborns in the treated groups with different doses of CeO_2 was not significantly different compared with the control group. Also, the testicular weight of the 2- and 6-day-old newborns of the treated groups with different doses of CeO_2 did not show any significant difference compared with the control group (Table 2).

Comparison of testicular volume in the 2-day-old newborn shows a slight increase in testicular volume at low doses (10 mg/kg.bw CeO_2) compared with the control group, but there was a significant decrease in the high-dose group

(250 mg/kg.bw CeO_2) compared with the control group ($P < 0.025$). Changes in testicular volume were not significant in the 6-day-old newborn (Table 2).

Previous studies have confirmed our findings. According to the studies done into the effect of cerium on body weight and volume, it has been shown that its effect depends on dose and time and also its effect is different in various organs and animals [1, 40, 41].

Conclusions

Our study was the first study that investigated the effect of *cerium(IV) oxide* on testicular tissues of neonatal mice. According to the changes in the number of testicular tissue cells and the biochemical evaluations of blood serum and testicular tissues of newborn mice, and also, changes in the weight and volume of the newborn testicular tissue, it can be concluded that receiving cerium oxide at high doses will be harmful for the development of testicular tissue. Also data from this study show that cerium oxide can act as an antioxidant for the tissue and cells if received at low and tolerable doses. Therefore, the adverse effects of CeO_2 observed in the male reproductive system of neonatal mice may be dose-dependent.

Acknowledgments Eventually, the authors of this article would like to thank Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences and Kermanshah University of Medical Sciences for funding, support, and cooperation in this project.

Compliance with Ethical Standards

The study was approved by the Institutional Animal Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran. Ethical clearance number for this study is IR.KUMS.REC.1397.1004.

Conflict of Interest The authors declare that they have no conflicts of interest.

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