Protective effects of Curcumin on testicular toxicity induced by titanium dioxide nanoparticles in mice

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ABSTRACT

Objective: In this study, we investigated the preventing effects of Curcumin (Cur) against titanium dioxide nanoparticle (NTiO2)-induced mouse testicular damage.

Methods: We assessed NTiO2-intoxicated mice received 50mg/kg of NTiO2, for 35 days. The Cur + NTiO2 group was pretreated with Cur (200 mg/kg) for 7 days prior to administering NTiO2. Sperm parameters, testosterone, concentration, histological criteria, morphometric parameters and Johnson's scoring.

Results: NTiO2 significantly reduced testicular weight, testosterone concentration, morphometric parameters, Johnson's scoring and sperm quality (p<0.01), as well as a significant increase in histological criteria. Pretreatment with Cur reduced testicular weight, ameliorated morphometric parameters, increased Johnson's scoring, elevated testosterone levels, and increased histological criteria such as vacuolization, detachment, and sloughing of germ cells into the seminiferous tubules. Cur also improved sperm parameters including sperm count, motility, and percentage of abnormality.

Conclusion: Cur was found to have a potent protective effect against spermatogenesis defects induced by nanoparticles in mice.

Keywords: curcumin, nanoparticle, spermatogenesis, testosterone, sperm quality, mice

INTRODUCTION

Many recent in vivo studies have shown that most nanoparticles (NPs) have toxic effects on male germ cells (Iavicoli et al., 2013; Brohi et al., 2017). NPs have the capacity to cross the blood-testis barrier and induce testicular toxicity (Lan & Yang, 2012). Among the various metal nanomaterials, titanium dioxide NPs (NTiO2) is used in a variety of consumer products such as sunscreens, cosmetics, clothing, electronics, paints and surface coatings (Cox et al., 2016). NTiO2 has been shown to cause reproductive toxicity including spermiation failure, low sperm production, and abnormal sperm morphology in mice (Miura et al., 2014). In vitro studies have also shown that NTiO2 induces cellular toxicity in the reproductive system (Hong et al., 2016). NTiO2 can be absorbed by spermatids, Sertoli cells, and Leydig cells, and induce histological changes in seminiferous tubules, causing damage to sperm production, sperm motility, and Sertoli cell number (Gao et al., 2013).

Since the high-rate use of NPs, such as NTiO2, affects male reproduction or male reproductive function, it seems crucial to find a suitable drug for reducing its toxic effects. Curcumin (Cur), a yellow pigment present in the rhizome of turmeric (Curcuma longa), has several pharmacological properties including anti-inflammatory, anti-carcinogenesis, antioxidant, and hypocholesterolemic activities (Hewlings & Kalman, 2017). Previous studies showed beneficial effects of Cur against testis damages. For example, Cur inhibited the testicular damage induced by alcohol, cisplatin, aflatoxin, metronidazole, ischemia reperfusion and cadmium (Giannessi et al., 2008; Ilbey et al., 2009; Mathuria & Verma, 2007; Noorafshan et al., 2010; Wei et al., 2009; Salama & El-Bahr, 2007). Therefore, we performed the present study to evaluate the preventive effects of Cur on NTiO2-induced testicular damage in the mouse.

MATERIAL AND METHODS

Animals

Thirty-two NMRI mice (6-8 weeks, 25-30 g) were used in this study. The mice were kept under a light-dark cycle of 12:12 at a temperature of 22 ± 3°C and a humidity of 50±5%. They were given free access to water and pellets (commercial food). Our study was approved by the Ethics committee of the Ahvaz Jundishapur University of Medical Sciences (approved number: IR.AJUMS.REC. 1393s98).

Experimental design

The study mice were randomly assigned to 4 groups (8 animals in each group) described below (Fig. 1):

Group 1 (Control): received normal saline (0.2 ml) for 42 days.

Group 2 (Cur): was treated with Cur (200 mg/kg) for 42 days (Al-Rubaei et al., 2014).

Group 3 (NTiO2): was administered first by normal saline (0.2 ml) for 7 days and then simultaneously with NTiO2 (50mg/kg) for 35 days (Song et al., 2017).

Group 4 (Cur + NTiO2): received first 200 mg/kg of Cur for 7 days followed by concomitant administration of 50mg/kg NTiO2, for 35 days.

All treatments were given by gavage. Twenty-four hours after the last administration, the mice were sacrificed, and the testicles from each animal were dissected, weighed and maintained in 10% formalin. Paraffin sections (5 µm) were prepared and stained with hematoxylin and eosin (H&E) for histology, Johnson's scoring and morphometric evaluations.

 Nanoparticle preparation

The NTiO2 (Sigma-Aldrich Co.) was diluted by milli-Q water (Song et al., 2017). A dynamic light scattering approach of Zetasizer–Nano-ZSP (Malvern, UK) was used to analyze the mean particle size distribution, polydispersity index (PDI) and zeta potential. The mean particle size was 68.4±5.7. The zeta potential value was measured to be +26.2 mV, high enough to make the NPs repel each other and prevent particle aggregation. The PDI value was calculated to be 0.19, showing an excellent homogeneous ZNP size distribution.
**Testosterone assay**

The blood samples were collected from the mice heart and centrifuged to obtain serum. Serum testosterone concentration was measured using a commercial Testosterone ELISA kit (SE120089, Sigma, St Louis, MO, USA) according to manufacturer’s instructions.

**Morphometry**

The diameters of the seminiferous tubules and the lumen diameter were measured using the Motic Images Plus 3.0 software. The height of the seminiferous epithelium was calculated by subtracting the lumen diameter from the tubules’ diameter. For each animal, 100 tubules were analyzed.

**Histology**

Six microscopy slides per animal were examined for signs of testicular damage, including detachment of spermatogenic cells from the basal lamina, sloughing of germ cells and vacuolization in the germinal epithelium. We calculated the average percentage of affected tubules for each feature.

**Assessment of spermatogenesis**

The maturity of the germinal epithelium was graded using the Johnsen’s scoring system (Johnsen, 1970), a simple method for the evaluation of spermatogenesis. In each mouse, 150 tubules were considered and a score ranging from 1 to 10 was given for each tubule. The tubules with complete inactivity were scored as 1 and those with maximum activity (at least 5 or more spermatozoa in the lumen) were scored as 10.

**Sperm parameters**

Spermatozoa were collected from the right epididymal cauda for examination of sperm motility using
the Computer Assisted Semen Analysis (CASA) system (Hamilton Thorne, USA). The sperms were scored as immotile if no movement was detected. The percentages of rapid progressive, slow progressive and no progressive sperms were evaluated in each sample. A suspension of spermatozoa (from the left epididymal cauda) was prepared and counted using a Neubauer hemocytometer. One drop of the suspension was smeared and observed under a light microscope for morphological assessments (Goodson et al., 2011; Hajshafiha et al., 2013).

**Statistical analysis**

The data analysis was performed using the SPSS (version 21.0, Chicago, IL, USA) one-way analysis of variance (ANOVA), followed by post-hoc pairwise comparison applying the Bonferroni or Mann-Whitney U test. The variables were tested in SPSS for normality distribution and homogeneity. Furthermore, a p-value of less than 0.05 was considered statistically significant.

**RESULTS**

**Weight change**

No significant differences in body weight were seen between the Control and Experimental groups. The weight of the testicles in the Cur group was slightly higher than that of the Control group. A significant reduction in testicular weight was found in the NTiO₂ group (p<0.05). In the NTiO₂ + Cur-treated animals, a significant increase in testicular weight in comparison with the NTiO₂-intoxicated mice (p<0.05) was observed (Table 1).

**Testosterone assay**

As shown in Figure 2, no significant changes were found between the Control and Cur groups in testosterone concentration. The testosterone concentration was significantly reduced in the NTiO₂-intoxicated animals (p<0.01). There was a significant increase in testosterone levels in the Cur + NTiO₂ group compared to those treated with NTiO₂ (p<0.01).

**Histology**

Testicular sections in both the control and Cur groups had a normal appearance. In the NTiO₂ group, disorganization of germ cell layers, detachment, sloughing and atrophy was found, and the histologic criteria were significantly increased (p<0.01). Cur administration could attenuate the criteria compared to the NTiO₂-intoxicated animals (Table 2 and Figure 4).

**Spermatogenesis Assessment**

Normal spermatogenesis was seen in both control and Cur groups. In the NTiO₂ group, abnormal spermatogenesis was found in several tubules of each section and the Johnsne score was significantly decreased when compared to the Control animals (p<0.01). In the Cur + NTiO₂ group, a few tubules showed maturity arrest and the mean Johnsne score was slightly lower than that in the Control group (Figure 5).

**Sperm parameters**

The number and motility of sperms in the Cur group were significantly higher than those in the Control were (p<0.05), while the abnormality percentage was slightly reduced in this group. NTiO₂ induced a significant reduction in all sperm parameters (p<0.01). Pretreatment with Cur substantially attenuated the number, abnormality and motility of the sperms in comparison with the NTiO₂-intoxicated animals (Table 3).

**DISCUSSION**

Data from our study showed that the NTiO₂ treatment significantly reduced testicular weight, testosterone concentration, morphometric parameters, and

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**Table 1. Testis and body weight of the Control and Experimental groups**

| Groups          | Body weight (g) | Testisweight (mg) | Testis/body (mg/g) |
|-----------------|-----------------|-------------------|--------------------|
| Control         | 30.2±1.7        | 114.6±13.9        | 3.81±0.16          |
| Cur             | 30.3±2.2        | 115.1±16.4        | 3.84±0.21          |
| NTiO₂           | 28.3±1.2        | 82.1±17.4*        | 2.9±0.20*          |
| Cur + NTiO₂     | 30.1±1.9        | 107.4±15.5†       | 3.56±0.12†         |

Values are expressed as mean ± SD for 8 mice. *p<0.05, †p<0.01; * and † symbols respectively indicate comparison to control and NTiO₂-intoxicated groups.

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**Figure 2. Testosterone assessment of control and experimental groups.** *p<0.05, †p<0.01; * and † symbols respectively indicate comparison to the control and NTiO₂-intoxicated groups.
Figure 3. Morphometric parameters of control and experimental groups. *p<0.01, †p<0.05; * and † symbols respectively indicate comparison to the control and NTiO₂-intoxicated groups.

Table 2. Histology assessments in the Control and Experimental groups

| Groups      | Percentage of tubules |
|-------------|-----------------------|
|             | Normal | Detached | Sloughed | Vacuolated |
| Control     | 94.5 ±4.9 | 1.3±0.5 | 1.1±0.2 | 4.6±0.5 |
| Cur         | 95.7±4.3 | 1.1±0.2 | 0.7±0.08 | 2.5±0.3 |
| NTiO₂       | 45.3±3.6** | 23.7±3.1** | 18.9±2.3** | 42.3±3.1** |
| Cur+NTiO₂   | 77.6±5.6** | 9.4±2.6** | 8.6±1.4** | 13.1±2.7** |

Values are expressed as mean ± SD for 8 mice. *p<0.05, **p<0.01, †p<0.01; * and † symbols respectively indicate comparison to control and NTiO₂-intoxicated groups.
sperm quality. NTiO₂ also induced histological changes and maturity arrest. Pretreatment with Cur could effectively attenuate these events.

The reduction in testicular weights of the NTiO₂-treated mice indicates the toxic effect of this nanoparticle on mouse testicles. The testicular weight is dependent on the germ cells mass. Thus, the reduction in testicular weight may be a consequence of germ cells death and spermatogenesis defects. In this study, Cur reversed testicular weight loss induced by NTiO₂, which is probably caused by the prevention of germ cell death in the seminiferous tubules.

The weight changes after NTiO₂ administration were accompanied by alterations in the morphometric parameters. In agreement with our results, Moridian et al. (2015) showed that zinc oxide NPs decreased seminiferous tubules’ diameter and seminiferous epithelium height. The decrease in seminiferous diameter may indicate germ cell loss induced by NTiO₂. The Cur-pretreatment could attenuate morphometric parameters indicated its beneficial effects on male germ cells. Mahmoudi et al. (2017) showed that Cur attenuated morphometric parameters of seminiferous tubules in sodium metabisulphite-treated mice. Cur ameliorated morphometric parameters in gentamicin-induced reproductive toxicity (Fetouh & El-Saied Azab, 2014). Ema et al. (2010) showed that NTiO₂ altered spermatogenesis and induced histological changes in the offspring’s testicles.

The remarkable decrease in morphometric parameters after NTiO₂ treatment was accompanied by histological changes to the seminiferous tubules, such as epithelial vacuolization, sloughing, detachment, and atrophy. These histological features are commonly observed in Sertoli cell damage after exposure to various toxicants (Russell & Griswold, 1995). Gao et al. (2013) showed that NTiO₂ aggregated in the Leydig cells, the Sertoli cells, and spermatids, which led to disruption of seminiferous tubules, reduction on the number of mature sperms, as well as decreased numbers of Sertoli cells in the mice. Zhao et
al. (2013) demonstrated that NTiO\textsubscript{2} induces apoptosis in Sertoli cells. Hong et al. (2016) showed that NTiO\textsubscript{2} induced cytotoxicity in primary cultured Sertoli cells of mice.

The Cur pretreatment effectively reversed vacuolization in the germinal epithelium, detachment of germ cells from basal lamina and sloughing of immature germ cells, indicating improvements in Sertoli cell functions. Cur increased the total number of Sertoli cells in gentamicin-induced reproductive toxicity in adult male guinea pigs (Fetouh & El-Saied Azab, 2014). Cur inhibited DNA fragmentation, apoptosis and cell cycle arrest induced by sodium arsenite in cultured murine Sertoli cells (Khan et al., 2013). Mahmoudi et al. (2017) also reported that Cur prevents structural changes in rat testicles induced by sodium meta-bisulfite.

The results of Johnsen's scoring also showed poor spermatogenesis in NTiO\textsubscript{2}-intoxicated animals. Alterations in the Johnsen scoring may relate to germ cell degeneration. The decreasing Johnsen score by Cur indicated the preventive effect of Cur on germ cell damage caused by NTiO\textsubscript{2}.

The administration of NTiO\textsubscript{2} significantly reduced testosterone concentrations, which was accompanied by a significant increase in histological criteria. Blanco-Rodríguez & Martínez-García (1998) reported that testosterone withdrawal induced detachment of germ cells from the seminiferous epithelium. Testosterone is required for the attachment of round spermatids to Sertoli cells (Smith & Walker, 2014). Komatsu et al. (2008) reported that NTiO\textsubscript{2} was absorbed by mouse Leydig TM3 cells and it affected testosterone secretion.

Pretreatment with Cur could raise the testosterone concentration by about 2.6-fold, higher than that of the NTiO\textsubscript{2}-intoxicated animals. Thus, Cur pretreatment may decrease genotoxicity and, consequently, reduce sperm abnormality. Karbalay-Doust & Noorafshah (2011) showed that Cur improved sperm abnormality in metronidazole-treated mice. The group pretreated with Cur also showed a significant increase in the percentage of rapid progressive and slow progressive sperms, and a significant decrease in the percentage of non-progressive and immotile sperms. On the other hand, the number of sperms in the Cur-pretreated groups was approximately 1.4-fold higher than those treated with NTiO\textsubscript{2}. These findings demonstrate that Cur pretreatment can improve sperm quality. Cur reduced metronidazole-induced testicular toxicity and improved the quality of sperms in mice (Noorafshah et al., 2010). Cur improved daily sperm production in gentamicin-induced reproductive toxicity in adult male guinea pigs (Fetouh & El-Saied Azab, 2014). The improved sperm quality in Cur pretreated mice was considered a result of improving testicular histology and elevation of testosterone concentration.

CONCLUSION

This study demonstrated that Cur improved the spermatogenesis defects in NTiO\textsubscript{2}-treated mice. This study suggests that Cur can protect germ cells by enhancing testosterone secretion. Further experiments are needed to clarify the mechanisms of the Cur effect on NP-induced toxicity. This study was conducted on mice and cannot be recommended for human clinical use. However, Cur has been suggested as a possible treatment for male reproductive disorders in humans exposed to high doses of nanoparticles.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 3. Sperm parameters in the Control and Experimental groups

| Parameters             | Control    | Cur        | NTiO\textsubscript{2} | NTiO\textsubscript{2} + Cur |
|------------------------|------------|------------|------------------------|-----------------------------|
| Sperm count (106/ml)   | 47.3±4.4   | 56.2±4.1*  | 26.4±4.1**             | 37.5±8.2**†                 |
| Abnormality (%)        | 23.1±2.4   | 21.3±2.1   | 63.7±5.2**             | 39.6±10.3**†                |
| Rapid progressive (%)  | 71.3±4.5   | 79.9±6.3*  | 23.2±3.1               | 68.6±4.5†                   |
| Slow progressive (%)   | 15.7±2.6   | 11.1±1.4   | 29.1±3.8**             | 14.9±0.24†                  |
| No progressive (%)     | 7.4±1.4    | 4.9±1.2    | 22.3±3.1**             | 10.8±0.36**†                |
| Immotile (%)           | 5.6±1.2    | 3.1±0.9    | 25.4±2.6**             | 5.7±0.16†                   |

Values are expressed as mean ± SD for 8 mice. *p<0.05, **p<0.01, †p<0.01.

* and † symbols respectively indicate comparison to the Control and NTiO\textsubscript{2}-intoxicated groups.
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