Original Article

The Impact of N-acetylcysteine Supplementation on Spermatogenic Recovery and Biochemical Markers in Dexamethasone-administered Mice

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ABSTRACT

Background: Dexamethasone (DEX), a widely used synthetic glucocorticoid, is associated with adverse effects on male reproductive health. N-acetylcysteine (NAC), as a glutathione precursor and potent antioxidant, helps neutralize free radicals, thereby preventing tissue damage and preserving sperm integrity.

Objectives: This study aimed to assess the effect of NAC on the undesired effects of DEX on the spermatogenesis indices and sperm parameters.

Methods: A total of 24 NMRI mice were divided into 4 groups: control, DEX (7 mg/kg/d, IP), NAC (100 mg/kg/d, IP), and DEX+NAC. They received treatments for 7 days. Testicular tissues were excised 24 hours after treatment completion for stereological evaluation and determination of mean daily sperm production (DSP). In addition, the mean numbers of spermatogonia, spermatocytes, spermatids, Leydig cells, and Sertoli cells, along with spermatogenesis indices, sperm tail length (STL), motility, and malondialdehyde (MDA) levels in serum and testicular tissues, and testosterone concentration were measured and analyzed.

Results: In the DEX-treated group, significant declines were detected in sperm motility, spermatogenesis indices, DSP, and the counts of spermatocytes, spermatids, and Leydig cells, along with a reduction in serum testosterone levels compared with the control ($P<0.05$). Conversely, serum and testicular MDA concentrations increased markedly following DEX administration ($P<0.05$). Co-treatment with NAC normalized these parameters to levels comparable to the control group.

Conclusion: The findings of this study suggest that NAC can mitigate the detrimental effects of DEX on spermatogenesis indices and sperm quality parameters, likely through its antioxidant and regulatory effects.

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Introduction

Male infertility and its reflection on reproduction have become a concern in recent years [1]. Toxins and medicines can interfere with the reproductive system [2]. Dexamethasone (DEX), a synthetic glucocorticoid, is widely prescribed in inflammatory disorders, autoimmune diseases [3], congenital hyperplasia of the adrenal glands, cerebral edema, shock, rheumatism, detecting Cushing syndrome, endogenous depression, as well as anti-nausea and anti-vomiting agents in patients undergoing chemotherapy [4]. It can suppress the immune system [5, 6], and also affects the hypothalamic-pituitary-gonadal hormone axis, causing significant changes in the level of sex hormones secretion [7, 8]. DEX induces oxidative stress, increases lipid peroxidation and reduces glutathione peroxidase in testicular tissue which ultimately inhibits testosterone secretion; reduce sperm count and motility and leads to a reduction in fertility rate [6, 9, 10]. N-acetylcysteine (NAC) is a derivative of the amino acid cysteine and a glutathione precursor, the major intracellular antioxidant, that decreases during oxidative stress. Therefore NAC consumption, as an antioxidant, can increase the level of glutathione [11], and can be effective in cardiovascular disease, human immunodeficiency virus infections, liver toxicity, and metal poisoning [12]. On the other hand, NAC can reduce apoptosis in male germ cells [13], decrease malondialdehyde (MDA) levels and increase glutamine peroxidation [14, 15]. It also improves sperm motility, semen volume, and viscosity [16]. The protective effects of NAC are mainly attributed to its function as a precursor for glutathione synthesis, which enhances intracellular antioxidant capacity and maintains redox homeostasis by neutralizing reactive oxygen species (ROS). In addition to this indirect effect, NAC directly scavenges free radicals, stabilizes mitochondrial function, and modulates apoptosis-related signalling pathways. By preventing oxidative damage and inhibiting pro-apoptotic cascades, NAC helps preserve testicular structure, supports the function of Leydig and Sertoli cells (SC), and promotes normal steroidogenic processes and spermatogenesis. Considering what was said, we aimed to investigate the influence of NAC, as an antioxidant compound, on toxic effects induced by DEX on spermatogenesis and histopathological alterations of testicular tissue in NMRI mice

Materials and Methods

Animals and treatments

In the present study, 24 adult male NMRI mice weighing 35±2 g were obtained from the Pasteur Institute of Iran and maintained under standard conditions (12 h light/12 h dark cycle, 21±2 ˚C) in the animal house of Arak University, with free access to food and water. The mice were randomly assigned to 4 study groups (each 6 mice): Control, DEX (IP injections with the dose of 7 mg/kg/d) (Sigma company, Germany), NAC (IP injections with the dose of 100 mg/kg/d) (Sigma company, USA), and DEX+NAC. Treatment lasted for 7 days. The selected doses of DEX [17-19] and NAC [20, 21] were based on previous studies.

Sample collection

Twenty-four hours after the final injection, the animals were weighed and humanely euthanized. The cauda epididymis was carefully excised, placed in 1.5 mL of Ham's F10 medium, and incubated at 37 ˚C for 5 min to obtain a sperm suspension for parameter evaluation. For biochemical analyses, blood samples were collected directly from the left ventricle, and their plasma was separated by centrifugation. The left testes were weighed, fixed in modified Davidson's fluid, and stained using the Heidenhain's azan method for stereological examination. The right testes were frozen in liquid nitrogen for 10 min and subsequently stored at −80 ˚C for the assessment of daily sperm production (DSP) and testicular MDA levels.

Evaluation of sperm motility

After pipetting, one drop of the sperm suspension was loaded onto a Neubauer counting chamber. The fields were studied using a light microscope at 200× magnification, and the percentage of motile, progressive motile, and immotile sperms were expressed according to the World Health Organization (WHO) recommendations [22].

Stereological study

The orientator technique was applied to prepare isotropic uniform random (IUR) sections of the testes. In this method, each testis was randomly positioned on a φ clock, which was divided into 9 equal sectors. A number between 1 and 9 was randomly selected, and a cut was made along the chosen line to divide the testis into two parts. The first half was then placed on a θ clock—divided into 9 unequal sectors—with its cut surface aligned

along the 0–0 axis. A second random number was selected, and another cut was made parallel to that number. The remaining portion from the second cut was placed perpendicularly on the θ clock so that its cut surface coincided with the 0–0 axis, followed by another parallel incision through a randomly selected number. After standard tissue processing, serial sections with thicknesses of 5 and 20 µm were obtained using a Leitz 1512 microtome and subsequently stained with the Heidenhain's azan technique [23, 24].

Shrinkage calculation

To determine the extent of testicular shrinkage, 3 randomly selected tissue samples were obtained from the IUR regions using a trocar. For each sample, two vertical diameters were measured, and their average was used to calculate the mean radius, referred to as the pre-fixation radius (rbefore). Following fixation, tissue processing, sectioning, and staining procedures, the mean radius of each sample was measured again and recorded as the post-fixation radius (rafter). The extent of testicular shrinkage for each specimen was then determined using the following equation and by applying it to the initial volume of the testicle (immersion volume), the absolute volume of the testis (Vtotal) was estimated [25] (Equation 1).

$$1.\ \text{Shrinkage} = 1 - \left(\frac{r_{after}^{\,2}}{r_{befor}^{\,2}} \right)^{\frac{3}{2}}$$

Calculating the number of spermatogenic, sertoli and leydig cells

The optical dissector technique along with an unbiased counting frame was applied to quantify spermatocytes, round and elongated spermatids, spermatogonia, as well as the Leydig and SCs. Random microscopic fields at 100× magnification were selected from tissue sections with a thickness of 20 µm, and a microcator (ND 221 B, Heidenhain, Germany) was utilized for the counting process. The numerical density (Nv) of various cell types was then calculated using the Equation 2.

$$2.\ Nv = \frac{\sum_{i=1}^{n} Q_i}{h \sum_{i=1}^{n} P_i \cdot {}^{a}/_{f}}$$

Where ΣQi is the total number of cells counted, h is the tissue thickness that is considered for counting, a/f is the area of the counting frame at the true tissue scale and ΣPi is the total number of points superimposed on the selected fields. Subsequently, to determine the absolute total number of cells (Ntotal), the numerical density (Nv) is multiplied by the total volume (Vtotal) (Equation 3):

3. Ntotal=Nv×Vtotal [25]

Evaluating the spermatogenesis index

Histological assessment was performed on 5-µm tissue sections using an Olympus BX41TE optical microscope (Japan) at 400× magnification. For each animal, all morphometric indices (spermiogenesis (SP), SC, meiosis (M), tubular differentiation indexes [TDI]) were quantified through systematic random sampling of 100 seminiferous tubule cross-sections.

The spermiogenesis index (SPI) represents the percentage of seminiferous tubules that contain mature spermatozoa. The SC index (SCI) describes the ratio between germ cells and SCs. The meiosis index (MI) indicates the proportion of round spermatids relative to primary spermatocytes. Finally, the TDI is defined as the percentage of seminiferous tubules that exhibit at least three different germ cell types derived from type A spermatogonia [18].

Estimating the sperm tail length (STL)

To measure STL, a sperm suspension was first prepared and stained with eosin-nigrosin. A drop of the stained sample was spread onto a glass slide and examined under a light microscope (Olympus BX41TE, Japan) equipped with a digital camera (Olympus DP12, Japan) at 400× magnification. Using a test grid, a total of 200 spermatozoa were evaluated. The STL was then determined through stereological techniques applicable to length estimation in two-dimensional (2D) images. Microscopic fields were selected using a systematic random sampling approach. In accordance with stereological principles, approximately 100–200 probe interactions were recorded, such as counting 100–200 sperm heads within the counting frame or 100–200 intersections of the Merz grid with sperm tails. The mean STL was subsequently calculated using the Equation 4:

4. $\Sigma L\ (total\ tails) = (\pi/2) \times (a/l) \times (1/asf) \times \Sigma I$

$L\ (tail) = \Sigma L / \Sigma N$

In the Merz grid, the constant "a/l" is determined as follows: The area of each basic tile on the grid is equal to X multiplied by Y. Therefore, the Merz grid constant "a/l" is calculated using the Equation 5:

5. $(X \times Y)/(\pi \times d)$

The term "asf" represents the ratio of the area of a basic tile to the area of the counting frame. "ΣI" denotes the total number of intersections between the sperm tails and the semicircles, while "ΣN" indicates the total number of sperms counted within the unbiased counting frame [24].

DSP

The right testes were first weighed and decapsulated, after which the testicular parenchyma was homogenized in 2 mL of NaCl solution containing 0.05% Triton X-100. To visualize the spermatids, a few drops of 1% eosin were added to the homogenate. Subsequently, 5 µL aliquot of the suspension was transferred to a Neubauer chamber, and the sperm cells were examined under a light microscope at 400× magnification.

The DSP was calculated according to the Equation 6:

6. $X= a\times5\times b\times10000$,

where "X" represents the number of spermatids per milliliter of sample, "a" is the count of spermatids in 5 squares, 5 is a constant, and "b" denotes the dilution factor.

The testicular spermatid number (TSN) was obtained using the Equation 7:

7. $(TSN)= X/W$

where W is the testicular weight (in g).

Finally, the DSP was determined as Equation 8:

8. $DSP= TSN/4.84$

where 4.84 corresponds to the average number of days required for developing spermatids to progress through the 14–16 cell stage of spermatogenesis in mice [18, 26, 27].

Biochemical studies

Evaluating Serum MDA

The serum MDA level was measured using the method described by Buege and Aust. For this purpose, a TCA–TBA–HCl reagent was prepared, consisting of 15% (g/mL) trichloroacetic acid (TCA), 0.375% (g/mL) thiobarbituric acid (TBA), and 25% normal hydrochloric acid (HCl). One part of the serum sample was mixed with two parts of this reagent, and the mixture was incubated in a water bath at 100 ˚C for 15 min. Following incubation, the samples were rapidly cooled in cold water and centrifuged for 10 min. The supernatant was then collected, and its absorbance was read at 532 nm using a spectrophotometer (T80C, PG Instruments Ltd, London, UK). The MDA concentration was determined from the absorbance values using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nmol/mL [28, 29].

Testicular MDA assay

The right testis was weighed and homogenized with KCl solution for 2 min. Then, it was mixed with a TBA-TCA solution. The reaction mixture was incubated in a water bath at 100 ˚C for 15 min, then rapidly cooled and centrifuged at 10000 g for 10 min. The absorbance of the resulting supernatant was measured at 532 nm using a spectrophotometer. The MDA content in the testicular tissue homogenate was quantified and expressed as nmol/mg of tissue [15].

Testosterone assay

Testosterone levels were measured using an enzyme-linked immunosorbent assay (ELISA) with the Monobind Testosterone ELISA kit (EIA-37K5J5, Monobind Inc., Lake Forest, California, USA), following the protocol provided by the manufacturer.

Statistical analysis

Data analysis was performed using SPSS software, version 16. One-way analysis of variance (ANOVA) followed by Tukey post hoc test was used to compare group means, with differences considered statistically significant at $P<0.05$.

Results

Histopathological findings

Histological studies of the testis tissue in the control group showed a normal testis tissue and the seminiferous tubules with normal spermatogenesis (Figure 1A). In the DEX group, decreased spermatogenesis and increased intertissue with edema were found. The testicular tissue of this group demonstrated marked degenerative changes, such as decreased germinal epithelial height, vacuolization, epithelial rupture in the seminiferous tubules, and reduced sperm accumulation within the tubular lumen (Figure 1B). Microscopic evaluation of the testes in the DEX+NAC group revealed that the majority of histopathological alterations induced by DEX were ameliorated, with the seminiferous tubules showing an almost normal epithelial configuration (Figure 1C). The NAC group demonstrated tubules structurally similar to those of the control group (Figure 1D).

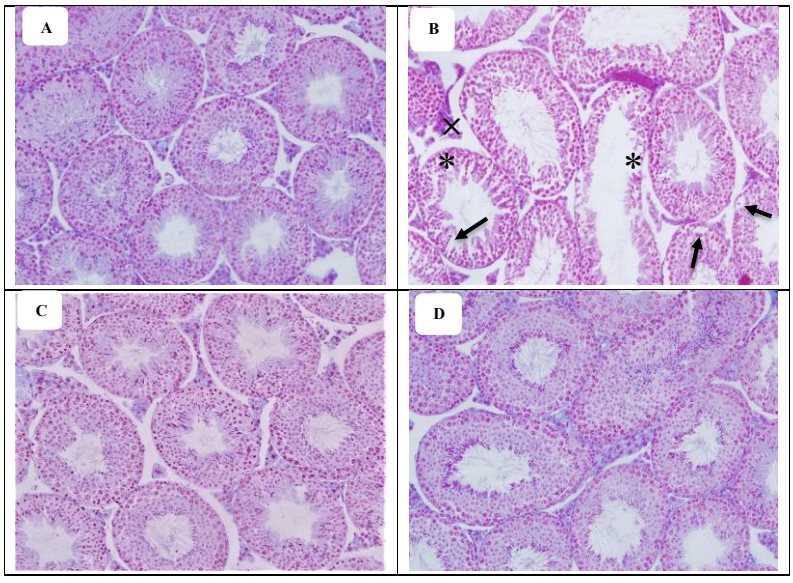

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Figure 1. Microscopic images of the testis tissue in adult mice in different experimental groups treated with DEX (7 mg/kg/d), NAC (100 mg/kg/d), and DEX+NAC (5 µm thick sections, stained with Heidenhain's azan method and magnification of ×200)

A) Control group representing the normal structure of the testis tissue; B) DEX group showing a reduction of the reproductive epithelium height (*), destruction of spermatogenesis, reduction of spermatocyte cells, irregularity and vacuolization (↗), increase and congest in interstitial tissue space (×); C) DEX+NAC group indicating an approximately normal seminiferous epithelial and spermatogenesis organization; D) NAC group showing the normal testicular histology similar to the control.

Body and testis weight

Statistical analysis showed no significant differences in the mean body weights betrween the control, NAC, DEX, and DEX+NAC groups after the treatment period (P>0.05). Also, the mean testis weight did not differ significantly between the experimental groups (P>0.05) (Table 1).

The mean number of spermatocytes, round and long spermatids, spermatogonia, leydig and SCs

In the DEX-treated group, the mean numbers of spermatocytes, round spermatids, elongated spermatids, and Leydig cells were significantly reduced compared with the other experimental groups (P<0.01). These parameters increased significantly in the DEX+NAC group to the control level (P>0.05). No significant differences were observed in the mean numbers of spermatogonia and SCs among the groups (P>0.05) (Table 2).

Table 1. Comparing the mean body and testis weight in different groups, 7 days after treatment with DEX (7 mg/kg/d), NAC (100 mg/kg/d), and DEX+NAC

Groups	Mean±SD		
	Primary Body Weight (g)	Final Body Weight (g)	Testis Weight (g)
Control	35.48±2.68[a]	36.25±1.85[a]	0.107±0.007[a]
DEX	35.45±2.37[a]	32.96±2.40[a]	0.1±0.008[a]
DEX+NAC	35.68±1.31[a]	35.5±1.92[a]	0.104±0.008[a]
NAC	35.13±2.86[a]	35.95±1.96[a]	0.109±0.008[a]

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Abbreviations: DEX: Dexamethasone; NAC: N-acetylcysteine; ANOVA: Analysis of variance.

[a]No statistically significant difference between groups (one-way ANOVA followed by Tukey's test, P≥0.05).

Table 2. Comparing the mean number of spermatocyte, round and long spermatid, spermatogonia, leydig and sertoli cells in different groups of mice after 7 days of treatment with DEX (7 mg/kg/d), NAC (100 mg/kg/d), and DEX+NAC

Groups	Mean±SD					
	Spermatogonia 10^6	Spermatocyte 10^6	Round Spermatid 10^6	Long Spermatid 10^6	Sertoli 10^6	Leydig 10^6
Control	6.03±0.27^a	27.35±0.96^a	38.18±3.04^a	35.89±2.26^a	3.96±0.58^a	2.88±0.18^a
DEX	5.5±0.35^a	17.96±0.88^b	26.29±2.24^b	20.93±1.44^b	3.89±0.46^a	2.11±0.09^b
DEX+NAC	5.77±0.26^a	26.33±0.62^a	36.64±2.98^a	34.87±1.02^a	3.91±0.33^a	2.73±0.18^a
NAC	6.27±0.28^a	27.02±1.11^a	38.24±2.96^a	35.93±1.55^a	4.02±0.26^a	2.96±0.18^a

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Abbreviations: DEX: Dexamethasone; NAC: N-acetylcysteine; ANOVA: Analysis of variance.

Significant differences between groups are indicated by different superscript letters, as determined by one-way ANOVA followed by Tukey test, P<0.05).

Spermatogenesis indices

The M (P<0.05), SP (P<0.05), and TD (P<0.003) indices showed a significant reduction in the DEX-treated group compared with the control group. In contrast, these parameters were markedly improved in the DEX+NAC group, reaching values comparable to those of the control. No significant difference was detected in the SCI among the four groups (P>0.05) (Table 3).

Sperm motility

A significant decline in the mean percentage of progressively motile sperm (P<0.001) and a concomitant rise in non-progressive and immotile sperm (P<0.001) were recorded in the DEX-treated group compared with the control. Co-administration of NAC with DEX significantly ameliorated these changes, resulting in values comparable to the control level (P>0.05) (Table 4).

STL

Comparing the mean STL revealed no significant difference between the DEX group and the control, NAC, and DEX+NAC groups (P>0.05) (Table 4).

DSP rate

A significant decline in the mean DSP was observed in the DEX group relative to the control group (P<0.01). In the DEX+NAC group, this parameter increased significantly, reaching values comparable to those of the control group (P>0.05) (Table 4).

Lipid peroxidation level

A significant increase in serum and testicular MDA concentrations was observed in the DEX group relative to the control group (P<0.001). Co-administration of NAC with DEX significantly reduced MDA levels, restoring them to control values (P>0.05) (Table 5).

Table 3. Comparing the mean values of TD, SP, M, and SC indices among different groups of mice following 7 days of treatment with DEX (7 mg/kg/d), NAC (100 mg/kg/d), and DEX+NAC

Groups	Mean±SD			
	TDI (%)	SPI (%)	SCI	MI
Control	92.83±5.45^a	87.66±6.94^a	26.53±0.97^a	2.35±0.01^a
DEX	81±3.74^b	72.83±2.99^b	26.01±0.95^a	2.01±0.01^b
DEX+NAC	87.66±5.27^a	87.5±5.35^a	26.47±0.93^a	2.43±0.03^a
NAC	92.9±5.17^a	87.83±4.99^a	26.89±0.83^a	2.36±0^a

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Abbreviations: TDI: Tubule differentiation index; SPI: Spermatogenesis index; SCI: Sertoli cell index; MI: Meiotic index; DEX: dexamethasone; NAC: N-acetylcysteine; ANOVA: Analysis of variance.

Note: Significant differences between groups are indicated by different superscript letters, as determined by one-way ANOVA followed by Tukey test (P<0.05).

Table 4. Comparing the mean values of sperm motility, sperm tail length, and DSP among different groups of mice following 7 days of treatment with DEX (7 mg/kg/d), NAC (100 mg/kg/d), and their combination (DEX+NAC)

Groups	Progressive Motility	Non-progressive Motility	Immotility	STL (µm)	DSP (10^6/g Testes)
Control	76.34±1.21[a]	3.83±1.72[a]	19.83± 0.98[a]	84.83±7.57[a]	25.6±2.04[a]
DEX	38±10.07[b]	5.5±0.54[b]	56.5±9.13[b]	72.33±9.37[a]	16.21±2.23[b]
DEX+NAC	74.67±1.03[a]	4.98±0.89[ab]	20.35±1.03[a]	75.17±8.51[a]	21.47±3.24[a]
NAC	81.17±1.16[a]	2.5±0.83[a]	16.33±0.81[a]	85.17±7.98[a]	24.06±1.9[a]

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Abbreviations: STL: Sperm tail length; DSP: Daily sperm production; DEX: Dexamethasone; NAC: N-acetylcysteine; ANOVA: Analysis of variance.

Note: Significant differences between groups are indicated by different superscript letters, as determined by one-way ANOVA followed by Tukey test (P<0.05).

Testosterone hormone

The testosterone level showed a significant decline in the DEX-treated group compared with the control (P<0.05), whereas co-administration of NAC with DEX significantly increased testosterone levels, reaching values not significantly different from those of the control (P>0.05) (Table 5).

Discussion

Our findings revealed that DEX treatment in adult mice significantly decreased the mean number of spermatocytes, round and long spermatids, and Leydig cells, along with reductions in the indices of M, SP and TD, which is consistent with findings reported in previous studies [18, 30, 31]. DEX appears to exert its deleterious effects on testicular function through the activation of the Fas/FasL signalling pathway, stimulation of caspase activity, and upregulation of pro-apoptotic proteins such as Bax. Furthermore, DEX can induce mitochondrial membrane depolarization and increase the production of ROS, leading to oxidative stress. These alterations ultimately contribute to impaired steroidogenesis and a reduction in testosterone levels [10, 31–34].

It has been shown that high doses of DEX can result in apoptosis of SCs [17, 35]. However, in the present study, the mean number of spermatogonia, SCs and SCI in both DEX and control groups showed no significant differences, which is in accordance with the results obtained by Jorge and et al. [17]. This result could be due to the low-dose of DEX used in the current research or the short-term treatment or even the higher weight of mice studied in our case.

The DEX group exhibited a significantly lower proportion of progressively motile spermatozoa than the control group. Notably, the pronounced elevation in the proportions of non-progressive and immotile spermatozoa was detected in the DEX-treated mice relative to controls, which aligns with the results of previous research [36, 37]. DEX induces membrane lipid peroxidation through the production of free radicals [38], lead-

Table 5. Comparing the mean serum and tissue levels of MDA and testosterone among different groups of mice following 7 days of treatment with DEX (7 mg/kg/d), NAC (100 mg/kg/d), and their combination (DEX+NAC)

Group	Testes MDA (nmol/mg)	Serum MDA (nmol/ml)	Testosterone (ng/mL)
Control	8.85±0.63[a]	3.54±0.39[a]	0.97±0.09[a]
DEX	14.58±1.5[b]	6.16±0.09[b]	0.49±0.07[b]
DEX+NAC	9.89±1.09[a]	4±0.69[a]	0.79±0.10[a]
NAC	8.82±0.75[a]	3.71±0.12[a]	1.08±0.09[a]

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Abbreviations: MDA: Malondialdehyde; DEX: Dexamethasone; NAC: N-acetylcysteine; ANOVA: Analysis of variance.

Significant differences between groups are indicated by different superscript letters, as determined by one-way ANOVA followed by Tukey test (P<0.05).

ing to a loss of membrane fluidity, reduced activity of membrane-associated enzymes and ion channels, receptor inactivation, and nonspecific ion permeability, which can explain the gradual loss of sperm motility [39]. ROS production not only reduces the phosphorylation of axonal proteins but also damages the mitochondrial membrane resulting in the ATP depletion, which is the source of energy for sperm motility [39] ultimately leading to poor sperm motility [40]. Despite the reduction in sperm motility, our data showed no significant difference in the STL between the DEX and control groups, which is in accordance with the findings of our previous study [18].

A significant decline in the DSP rate was observed in mice treated with DEX relative to the control group, which has been reported by our previous study [18]. Generally, DSP depends on the number of somatic cells, including Sertoli and Leydig cells, and also the number of spermatogenic cells [41]. The Leydig cells are also responsible for providing the blood testosterone levels [42], which is crucial for the development and differentiation of sperm cells during spermatogenesis; Therefore, it can be inferred that DEX disrupts the process of spermatogenesis and reduces DSP by inducing apoptosis in spermatogenic, Sertoli, and Leydig cells [19]. Accumulating evidence along with the results of this study have also shown that DEX can lead to a decrease in the blood testosterone levels [10, 18, 43, 44] not only by disturbing the hypothalamus and the pituitary axis, but also by directly affecting the testis Leydig cells via transcriptional inhibition of genes involved in the enzymatic pathways of testosterone synthesis [44, 45], which could be considered another cause for the reduction found in the DSP in this study.

The findings of this study also demonstrate a significant increase in serum and testicular MDA levels in the DEX-treated group relative to controls, presumably resulting from oxidative stress [38] and increase of lipid peroxidation, these findings are in line with the previous researches [17, 18, 46].

Based on the results, no significant differences were observed in the mean testicular or body weights between the groups at the end of the treatment period. Some studies confirm our results [17, 47]; however, others have reported a reduction in testis [18] and body weight [48] in DEX-treated mice. These different outcomes might be related to the stage of treatment which could be either before or after puberty, the duration of treatment, mice strain, and the consumed dose of DEX.

NAC increases the levels of intracellular glutathione and antioxidant enzymes [49] and reduces ROS production [50] which contributes significantly to safeguarding cellular structures against oxidative injury and lipid peroxidation [51]. It can also suppress the expression of pro-apoptotic genes [14] by inhibiting the activities of c-Jun N-terminal kinase, MAP kinase p38, SAPK/INK, c-fos pathway, and NF-κB, thereby preventing cell death [52]. All this together can prevent testis tissue damage, apoptosis and degeneration of germ and Leydig cells [53], which explains the obtained results in this study where NAC significantly increased the number of germ, Sertoli and Leydig cells, spermatogenesis indices, DSP, sperm motility, testosterone levels and also decreased the serum and testis level of MDA to the control level in mice treated with DEX.

Conclusion

In the present study, NAC ameliorated the detrimental effects of DEX on spermatogenesis indices, sperm characteristics and DSP due to its antioxidant properties and its ability in increasing the levels of glutathione and testosterone. Therefore, the consumption of NAC in therapeutic regimens containing DEX is suggested.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Arak University of Medical Sciences, Arak, Iran (Code: IR.ARAKMU.1397.091).

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Authors' contributions

Data analysis: Monireh Mahmoodi and Malek Soleimani Mehranjani; Writing, review and editing: Monireh Mahmoodi; Manuscript revision: Malek Soleimani Mehranjani; Methodology, data collection and validation, drafting the manuscript, and laboratory experiments: Sepideh Bakhshi.

Conflict of interest

The authors declared no conflict of interest.

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