Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2025.71654

Turk J Obstet Gynecol 2025;22(3):266-74

Protective effect of N-acetylcysteine in doxorubicin-induced primary ovarian failure in female rats

Dişi sıçanlarda doksorubisin kaynaklı primer yumurtalık yetmezliğinde N-asetilsisteinin koruyucu etkisi

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Abstract

Objective: N-acetylcysteine (NAC), an aminothiol compound, eliminates free radicals and enhances glutathione (GSH) synthesis, thereby strengthening intracellular antioxidant defenses. Although its protective effects against ovarian injury have been reported, its efficacy in doxorubicin (DOX)-induced ovarian failure has not been demonstrated. This study aimed to investigate whether NAC exerts a protective role against DOX-induced ovarian toxicity in female rats.

Materials and Methods: Twenty-one adult female rats were randomly assigned to three groups: Control, DOX (10 mg/kg, i.p., single dose), and DOX+NAC (150 mg/kg, i.p., for 5 days; DOX administered on day 3, one hour after NAC). Serum and tissue oxidative stress parameters, histopathological changes, proliferating cell nuclear antigen (PCNA) immunoreactivity, and TUNEL assay were evaluated.

Results: DOX significantly reduced serum anti-Müllerian hormone (AMH) (6.75 → 5.31 ng/mL; p<0.001) and GSH (422.64 → 280.98 mg/L; p<0.001), while increasing tumor necrosis factor alpha (TNF-α) (175.87 → 260.77 ng/L; p<0.001) and total oxidant status (TOS) (7.18 → 11.84 U/mL; p=0.002). NAC treatment reversed these alterations, namely: AMH (6.51 ng/mL; p=0.004), GSH (363.86 mg/L; p=0.018), TNF-α (184.55 ng/L; p<0.001), TOS (7.88 U/mL; p=0.003). In ovarian tissue, DOX reduced GSH (123.63 → 80.64 mg/L; p=0.001) and total antioxidant status (14.88 → 10.57 U/mL; p<0.001), while elevating TOS (7.14 → 12.64 U/mL; p<0.001) and caspase-3 (2.06 → 3.14 ng/mL; p<0.001). NAC significantly improved all these parameters (p≤0.005). Histologically, DOX caused edema, hemorrhage, infiltration, and a reduction in the percentage of healthy follicles, whereas NAC markedly ameliorated these alterations. Furthermore, NAC enhanced PCNA expression and reduced TUNEL-positive granulosa cells, supporting its anti-apoptotic effect.

Conclusion: NAC preserved ovarian reserve and follicular integrity by suppressing oxidative stress, inflammation, and apoptosis induced by DOX. These findings highlight NAC as a promising protective agent against chemotherapy-induced ovarian toxicity.

Keywords: Doxorubicin, infertility, N-acetylcysteine, ovarian function, primary ovarian failure

PRECIS: N-acetylcysteine (NAC), a potent antioxidant and glutathione precursor, demonstrated significant protection against doxorubicin (DOX)-induced ovarian toxicity in a rat model. NAC preserved ovarian reserve and follicular integrity by attenuating oxidative stress, inflammation, and apoptosis, thereby restoring serum anti-Müllerian hormone and glutathione levels and reducing tumor necrosis factor alpha, total oxidant status, and caspase-3 activity. Histopathological and immunohistochemical analyses further confirmed enhanced follicular survival, reduced granulosa cell apoptosis, and increased proliferative capacity. These findings provide the first comprehensive evidence that NAC mitigates DOX-related ovarian failure, underscoring its translational potential as an adjuvant for fertility preservation in patients undergoing chemotherapy.

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Received/Geliş Tarihi: 22.07.2025 **Accepted/Kabul Tarihi:** 21.08.2025 **Epub:** 25.08.2025 **Publication Date/Yayınlanma Tarihi:** 05.09.2025

Cite this article as: Aslan İÖ, Öz M, Erdal H, Karaboğa İ, Doğan M. Protective effect of N-acetylcysteine in doxorubicin-induced primary ovarian failure in female rats. Turk J Obstet Gynecol. 2025;22(3):266-74

Öz

Amaç: N-asetilsistein (NAC), serbest radikalleri temizleyen ve glutatyon (GSH) sentezini artırarak antioksidan savunmayı güçlendiren bir aminotiyol türevidir. Yumurtalık hasarına karşı koruyucu etkileri bildirilmiş olsa da, doksorubisin (DOX) kaynaklı over yetmezliğinde etkinliği gösterilmemiştir. Bu çalışmada, NAC'nin DOX ile oluşturulan over hasarına karşı koruyucu etkisi araştırılması hedeflenmiştir.

Gereç ve Yöntemler: Yirmi bir erişkin dişi sıçan üç gruba ayrıldı: Kontrol, DOX (10 mg/kg, i.p., tek doz) ve DOX+NAC (150 mg/kg, i.p., 5 gün; 3. gün DOX, NAC'den 1 saat sonra). Serum ve doku oksidatif stres parametreleri, histopatolojik değişiklikler, çoğalan hücre nükleer antijeni (PCNA) immünoreaktivitesi ve TUNEL apoptoz analizi yapıldı.

Bulgular: DOX, serum anti-Müllerian hormone (AMH) (6,75 → 5,31 ng/mL; p<0,001) ve GSH'yi (422,64 → 280,98 mg/L; p<0,001) azaltırken; tümör nekroz faktörü alfayı (TNF-α) (175,87 → 260,77 ng/L; p<0,001) ve toplam oksidan kapasitesini (TOS) (7,18 → 11,84 U/mL; p=0,002) artırdı. NAC tedavisi bu değişiklikleri düzeltti: AMH (6,51 ng/mL; p=0,004), GSH (363,86 mg/L; p=0,018), TNF-α (184,55 ng/L; p<0,001), TOS (7,88 U/mL; p=0,003). Doku analizinde DOX, GSH'yi (123,63 → 80,64 mg/L; p=0,001) ve TOS'unu (14,88 → 10,57 U/mL; p<0,001) düşürürken; TOS (7,14 → 12,64 U/mL; p<0,001) ve kaspaz-3'ü (2,06 → 3,14 ng/mL; p<0,001) yükseltti. NAC tüm parametrelerde anlamlı iyileşme sağladı (p≤0,005). Histolojik olarak DOX, ödem, hemoraji ve enflamasyonu artırıp sağlıklı folikül oranını azaltırken; NAC bu bulguları düzeltti. Ayrıca PCNA'yı artırıp TUNEL pozitif hücreleri azalttı.

Sonuç: NAC, DOX'in neden olduğu oksidatif stres, enflamasyon ve apoptozu baskılayarak over rezervi ve folikül sağlığını koruyarak; kemoterapiye bağlı over toksisitesine karşı güçlü bir koruyucu ajan potansiyeli sunmuştur.

Anahtar Kelimeler: Doksorubisin, kısırlık, N-asetilsistein, yumurtalık fonksiyonu, primer yumurtalık yetmezliği

Introduction

Ovarian toxicity and infertility are major side effects of cancer treatment among pre-pubertal, adolescent and young adult female cancer patients. The ovary consists of follicles at various stages of development, with the follicles serving as the basic functional unit. There are a limited number of primordial follicles that form immediately after birth, and these follicles remain in a dormant state, representing the ovarian reserve, an indicator of fertility potential. With advancing age, primordial follicles are activated in regular waves and develop into growing follicles that include primary, secondary and antral stages to support hormone secretion, oocyte maturation and ovulation from birth until menopause, when the pool of primordial follicles is depleted[1].

Doxorubicin (DOX) is an antineoplastic drug used to treat various tumors, including leukaemia, lymphomas and soft tissue sarcomas[2]. However, the use of DOX may cause loss of primordial follicles and ovarian function, which may lead to depletion of ovarian reserve and consequently premature ovarian failure[1]. The toxic effects of DOX on the ovary have been associated with decreased antioxidant capacity and increased production of reactive oxygen species (ROS), mitochondrial damage, and inflammatory response, leading to cell apoptosis[3,4]. Administration of natural compounds with antioxidant properties may be a potential strategy to prevent or mitigate DOX-induced ovarian damage[5,6].

The use of antioxidants is steadily increasing as they retain important cytoprotective potential. One effective antioxidant compound is N-acetylcysteine (NAC). It is one of the oldest and most potent mucolytics, the preferred antidote in paracetamol poisoning, and an aminothiol compound[7]. The well-known antioxidant properties of NAC are attributed to its ability to scavenge free radicals and restore intracellular antioxidant defense by increasing glutathione (GSH) production through deacetylation to cysteine, the building block and rate-limiting step in GSH synthesis[8]. In terms of female reproduction, NAC has protective activity against ovarian damage caused by ischemia/reperfusion injury[9]. At the same time, NAC showed protective activity by decreasing the production of ROS and increasing GSH production in cisplatin-induced primary ovarian failure[10]. In gamma radiation-induced ovarian failure, NAC normalized anti-Müllerian hormone (AMH) levels and improved the histopathological and ultrastructural changes induced by γ radiation[11]. However, to the best of our knowledge, there is no study in the literature showing that NAC can protect ovarian function impaired by DOX. Therefore, this study was performed to investigate the protective efficacy of NAC, a GSH precursor, in ovarian failure by focusing on DOX-induced oxidative stress, apoptotic cascade, and inflammation in female rats induced by DOX.

We hypothesized that NAC would attenuate DOX-induced ovarian toxicity by reducing oxidative stress, inflammation, and apoptosis. The primary endpoint was preservation of healthy follicles and serum AMH levels, and secondary endpoints included histopathological injury scores, proliferating cell nuclear antigen (PCNA), TUNEL, and serum/tissue biomarkers [GSH, total antioxidant status (TAS), total oxidant status (TOS), tumor necrosis factor alpha (TNF-α), and caspase-3].

Materials and Methods

Ethical Approval

The current study followed the "Principles of laboratory animal care" (NIH publication no: 86-23, revised 1985) and, as well as the Aksaray University Experimental Animals Ethics Committee, and it was carried out in compliance with ethical standards (approval number: 14, date: 19.02.2024).

Animals and Study Groups

In a five-day study period, twenty-one adult female rats of the same age were randomly assigned to three groups (n=7 per group).

Control Group: Received no drug treatment, with saline administered intraperitoneally (i.p.) as a placebo.
DOX Group: Received a single dose of DOX (10 mg/kg, i.p.) on the third day of the study.
DOX+NAC Group: NAC (150 mg/kg, i.p.) was administered daily for five days. On the third day, DOX (10 mg/kg, i.p.) was injected one hour after NAC administration.
Since there are also studies in the literature in which the prophylactic drug alone was not administered in this model, only the NAC group was not included in this project[12,13].
It is observed that the estrous cycles of female rodents were not evaluated before starting the study. For this reason, healthy female rats of the same age were used in our project, and their cyclic status was ignored[12-14].
Determination of the Dose of DOX: The DOX dose was set at 10 mg/kg single dose based on the findings of a previous study[5]. There is evidence that this dosage schedule results in ovarian failure.
Determination of NAC Dose: The dose of NAC was set at 150 mg/kg based on the findings of a previous study[10].
Sample Collection: 24 hours after the last pharmacological drug administration*, 5 mL of blood was collected from all rats under anaesthesia. The serum was separated and stored at -80 °C until further study. Subsequently, they were euthanised by cervical dislocation and ovarian tissues were collected. One ovary of each rat was stored for biochemical analyses and the other for histopathological analyses[5].

Outcome Measures

Histomorphometric and Histopathogical Analysis

At the end of the experimental period, ovarian tissue (left ovary per animal) was fixed in 10% formalin for 48 h at room temperature. Samples were then washed in tap water, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax by standard histological method. Tissue blocks were sectioned at 5 µm thickness on a rotary microtome (Leica RM2245, Leica Biosystems, Germany) for histomorphometric, immunohistochemical, and apoptotic examinations.
Serial sections were stained with hematoxylin and eosin (H&E) to examine the histopathological changes in the ovarian tissue and to determine the ovarian reserve. Hemorrhage, edema, and infiltration histopathological findings were evaluated and scored for each criterion using a scale ranging from 0 to 3 (0; none, 1; mild, 2; moderate, and 3; severe)[15]. To determine ovarian follicular activity, follicles in each tissue were classified and recorded as primordial, primary, secondary, antral, and atretic. Healthy follicles (%) was calculated as previously described, (primordial+primary+secondary+antral follicles/total number of follicles x 100)[16].

Detection of PCNA Immunoreactivity

Immunohistochemical anti-PCNA immunoreactivity was evaluated by the indirect immunohistochemical method[17]. Tissue sections were deparaffinised in xylene, hydrated in graded ethanol, and washed with distilled water. Slides were washed in phosphate buffered saline, and boiled in citrate buffer using a microwave oven for antigen retrieval. To block non-specific binding, the slides were incubated with goat serum for 30 min. Then, slides were incubated with anti-PCNA primary antibody in humidified chamber for 1 hour. Subsequently, slides were incubated with biotinylated secondary antibody and streptavidin peroxidase for 30 min at room temperature. 3,3'-diaminobenzidine (DAB) was used as a chromogen to visualize nuclear PCNA immunoreactivity (brown staining). Counterstain was performed using Mayer's haematoxylin. For PCNA scoring, granulosa cells in follicles, were evaluated according to their DAB-positive staining in three distinct fields from each ovarian section. The number of granulosa cells staining PCNA-positive for each ovarian tissue was calculated as a percentage[18].

Detection of Follicular Apoptosis

The TUNEL staining method (Merck Millipore, Apoptag® Peroxidase *In Situ* Apoptosis Detection Kit, Darmstadt, Germany) was used to determine follicular apoptosis. The staining protocol was applied according to the manufacturer's instructions. Mayer's hematoxylin was used for counterstaining. From each ovarian section, TUNEL-positive granulosa cells and all granulosa cells, in the follicles were counted in 5 randomly selected high-power fields at 200x magnification. The percentage of TUNEL-positive granulosa cells (%) was determined by the formula, (number of TUNEL-positive granulosa cells / number of all granulosa cells) x 100[1].

Biochemical Analysis

To evaluate oxidative stress, inflammation, and ovarian function, several biochemical parameters were quantified both in serum and ovarian tissue. Specifically, TNF-α (BTLAB; Cat. No. E0764Ra), AMH Cat. No. E0456Ra, TAS Cat. No. E1710Ra, TOS Cat. No. E1512Ra, GSH Cat. No. EA0113Ra were measured in serum, while TNF-α Cat. No. E0764Ra, TAS Cat. No. E1710Ra, TOS Cat. No. E1512Ra, GSH Cat. No. EA0113Ra, and caspase-3 (BTLAB; Cat. No. E1648Ra) were analyzed in ovarian tissue. All analyses were performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions. The sandwich ELISA method was applied for TAS and TOS. The standard curve range of these kits is 0.02-60 U/mL, and their sensitivity is 0.013 U/mL. The samples were incubated at 37 °C for 60 minutes, washed five times, and then the substrate solutions were incubated in the dark at 37 °C for 10 minutes before being read at 450 nm. For GSH analysis, a competitive ELISA method was used. The standard curve range for this kit is 20-1600 mg/L, and its sensitivity is 10.25 mg/L. The sample and biotinylated antigen were incubated at 37 °C for 60 minutes, followed by avidin-HRP incubation at 37 °C for

60 minutes. After the washing steps, the substrate solutions were incubated in the dark at 37 °C for 10 minutes, and the absorbance was measured at 450 nm.

Statistical Analysis

Data were analyzed with SPSS (Version 23.0). The conformity of the data with normal distribution was evaluated with the Kolmogorov-Smirnov test. Comparisons of the groups were carried out using the Kruskal-Wallis test and the Mann-Whitney U test. Data were presented as mean ± standard deviation. Values of $p<0.05$ are statistically significant.

Results

Histopathological and Histomorphometric Findings

H&E-stained ovarian sections and histopathology scores of the groups are presented in Figure 1. It was observed that the ovarian tissue in the control group exhibited normal histological structure. The control group ovarian tissue cortex included primordial, primary, secondary, and antral follicles belonging to different stages of follicle development, and the stroma between them. The medulla structure of the control group exhibited normal loose connective tissue. In the DOX group, in addition to hemorrhage in the cortex and medulla, vascular congestion, edema, and infiltration were observed

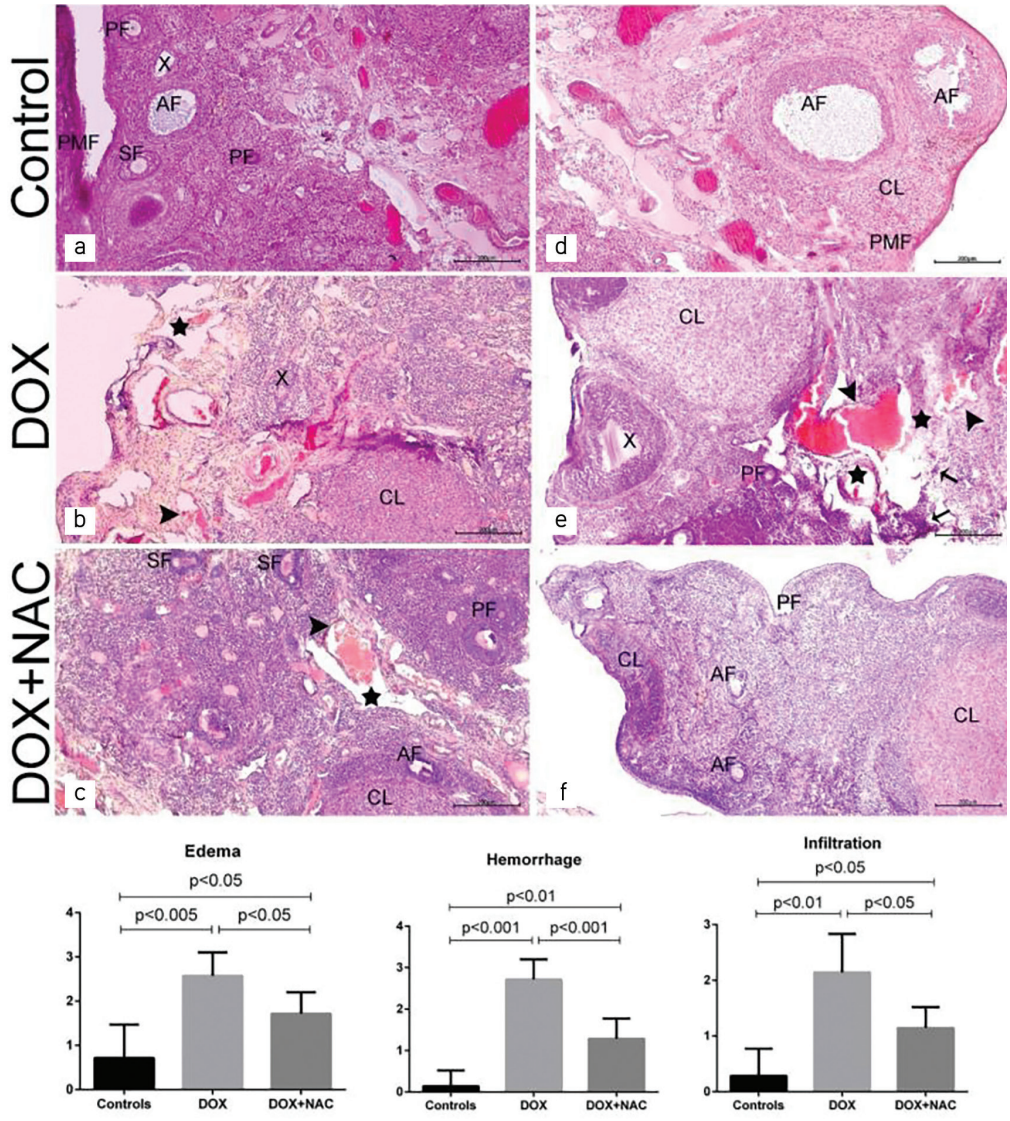

Figure 1. Effect of NAC-treatment on DOX-induced histopathological changes in ovarian tissue. Representative images of **(a)** control, **(b)** DOX, **(c)** DOX+NAC groups and the histopathological changes regarding **(d)** edema, **(e)** hemorrhage, **(f)** infiltration levels

PMF: Primordial follicle, PF: Primary follicle, SF: Secondary follicle, AF: Antral follicle, X: Atretic follicle, CL: Corpus luteum, Star: Edema, Arrowhead: Hemorrhage, Arrow: Infiltration, Dye: Hematoxylin-eosin, 100X, DOX: Doxorubicin, NAC: N-acetylcysteine

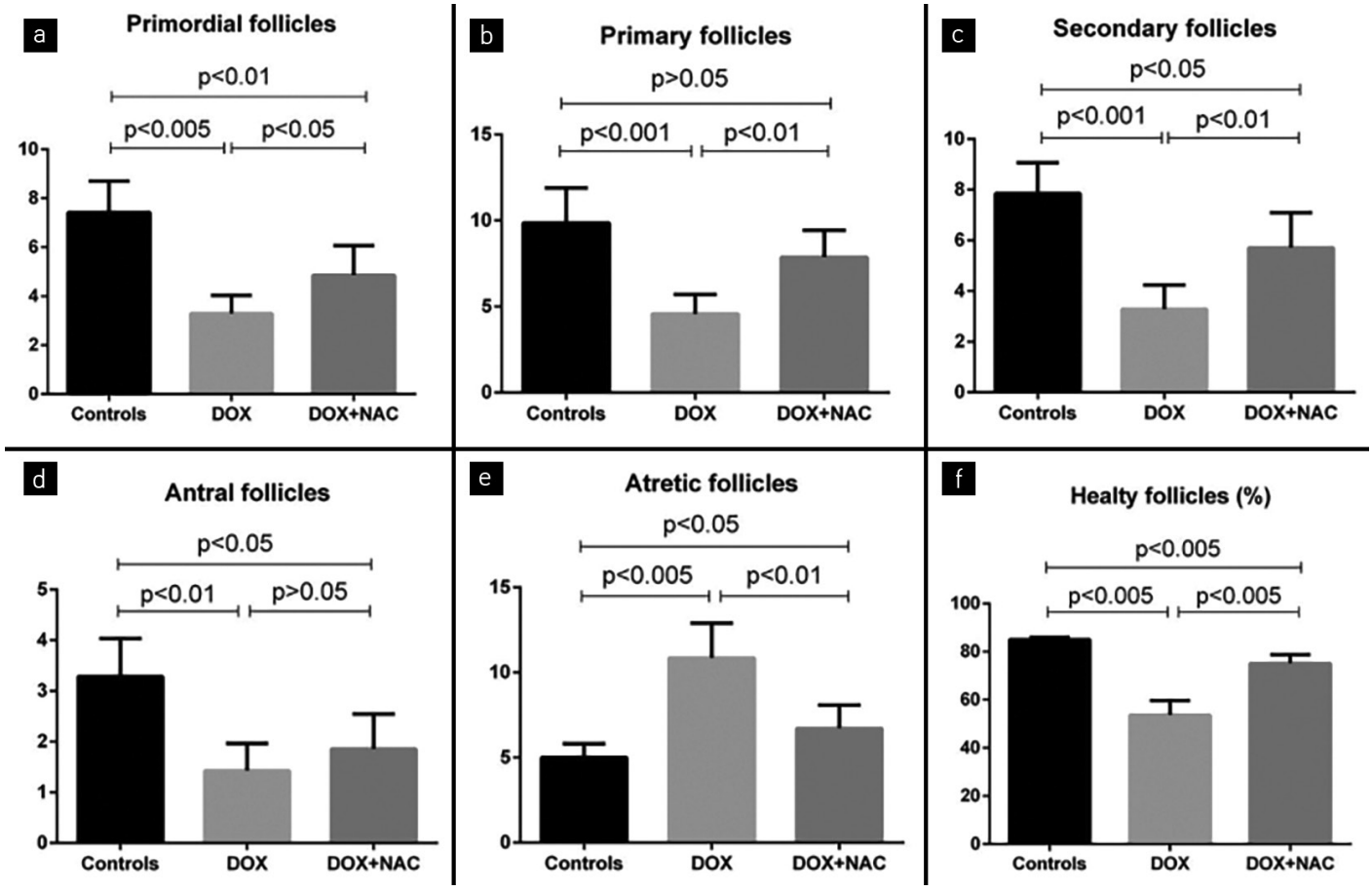

Figure 2. Effect of DOX and NAC-treatments on ovarian follicle numbers; **(a)** primordial, **(b)** primary, **(c)** secondary, **(d)** antral, **(e)** antretic follicles, and **(f)** healthy follicle percentage in control, DOX, DOX+NAC groups

DOX: Doxorubicin, NAC: N-acetylcysteine

in the medulla. Compared to the control group, the scores of edema, hemorrhage, and the infiltration increased score increased significantly in the DOX group. It was determined that these histopathological changes were significantly alleviated in the DOX + NAC group compared to the DOX group.

The percentage of healthy follicles and ovarian reserve of all groups is presented in Figure 2. The number of primordial, primary, secondary, and antral follicles was significantly lower in the DOX group than in the control group. The number of atretic follicles was significantly higher in the DOX group compared to the control group. A significant increase in the number of primordial, primary, secondary, and antral follicles was detected in the NAC treatment group compared to the DOX group. Additionally, the percentage of healthy follicles decreased significantly in the DOX group compared to the control group; it increased significantly in the DOX+NAC group compared to the DOX group.

PCNA Immunoreactivity Findings

Findings of cell proliferation marker PCNA immunoreactivity in ovarian tissue are presented in Figure 3. PCNA immunoreactivity is observed in follicular granulosa cells in all groups. While PCNA immunoreactivity decreased in the DOX group compared to the control group, a significant increase in PCNA immunoreactivity was detected in the DOX+NAC group compared to the DOX group.

Follicular Apoptosis Findings

Findings of follicular apoptosis are presented in Figure 4. The percentage of apoptotic granulosa cells was significantly increased in the DOX group compared to the control group. Granulosa cell apoptosis was significantly reduced in the NAC treatment group compared to the DOX group[15,16].

Biochemical Analysis

As shown in Table 1, DOX treatment induced substantial oxidative stress, inflammation, and ovarian dysfunction, as evidenced by significant changes in AMH, GSH, TNF-α, TAS, and TOS levels. NAC co-treatment effectively mitigated these adverse effects, restoring oxidative balance and reducing inflammation, thereby demonstrating its protective potential in counteracting DOX-induced systemic and reproductive toxicity.

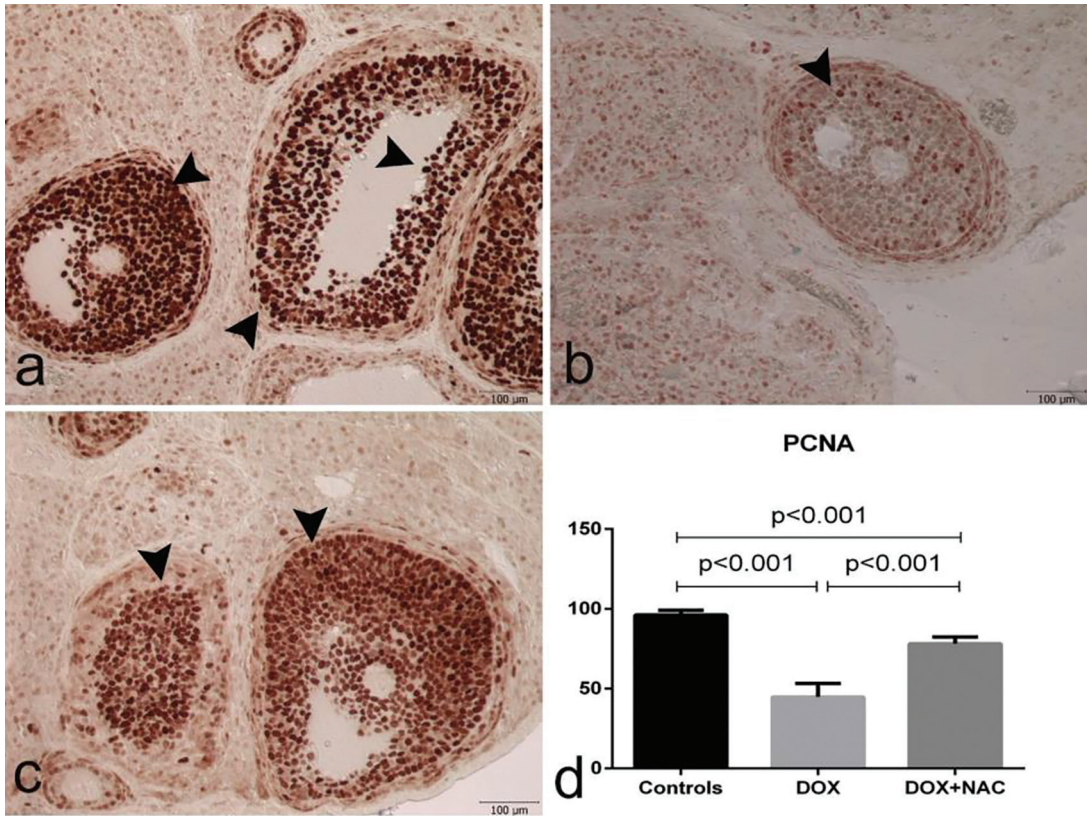

Figure 3. Effect of NAC and DOX treatments on PCNA immunoreactivity in granulosa cells of ovarian follicles. **(a)** control, **(b)** DOX, **(c)** DOX+NAC, **(d)** distribution of PCNA immunoreactivity of groups, arrowhead; PCNA-positive cells, magnification; 200X

DOX: Doxorubicin, NAC: N-acetylcysteine, PCNA: Proliferating cell nuclear antigen

Table 1. Serum biomarkers data

Serum biomarkers	Control (mean ± SD)	DOX (mean ± SD)	DOX+NAC (mean ± SD)	Control vs. DOX p-value	DOX vs. DOX+NAC p-value
AMH (ng/mL)	6.75±0.49	5.31±0.47	6.51±0.78	<0.001	0.003931087
GSH (mg/L)	422.64±69.23	280.98±32.26	363.86±73.71	<0.001	0.018416596
TNF-α (ng/L)	175.87±25.91	260.77±18.08	184.55±30.0	<0.001	0.000109148
TAS (U/mL)	8.13±1.01	6.12±1.03	7.56±0.59	0.001	0.003392724
TOS (U/mL)	7.18±1.00	11.84±2.55	7.88±1.12	0.002	0.003191524

SD: Standard deviation, AMH: Anti-Müllerian hormone, GSH: Glutathione, TNF-α: Tumor necrosis factor alpha, TAS: Total antioxidant status, TOS: Total oxidant status, DOX: Doxorubicin, NAC: N-acetylcysteine

Table 2. Ovarian tissue biomarkers

Ovarian biomarkers	Control (mean ± SD)	DOX (mean ± SD)	DOX+NAC (mean ± SD)	Control vs. DOX p-value	DOX vs. DOX+NAC p-value
GSH (mg/L)	123.63±20.75	80.64±15.44	118.57±23.04	0.001	0.005495828
TNF-α (ng/L)	194.73±26.61	289.47±21.29	230.82±31.50	<0.001	0.000955061
TAS (U/mL)	14.88±0.77	10.57±1.01	13.74±1.02	<0.001	0.000177902
TOS (U/mL)	7.14±0.62	12.64±1.12	9.03±0.58	<0.001	0.000000737
Caspase-3 (ng/mL)	2.06±0.29	3.14±0.33	2.17±0.28	<0.001	0.000108463

SD: Standard deviation, GSH: Glutathione, TNF-α: Tumor necrosis factor alpha, TAS: Total antioxidant status, TOS: Total oxidant status, DOX: Doxorubicin, NAC: N-acetylcysteine

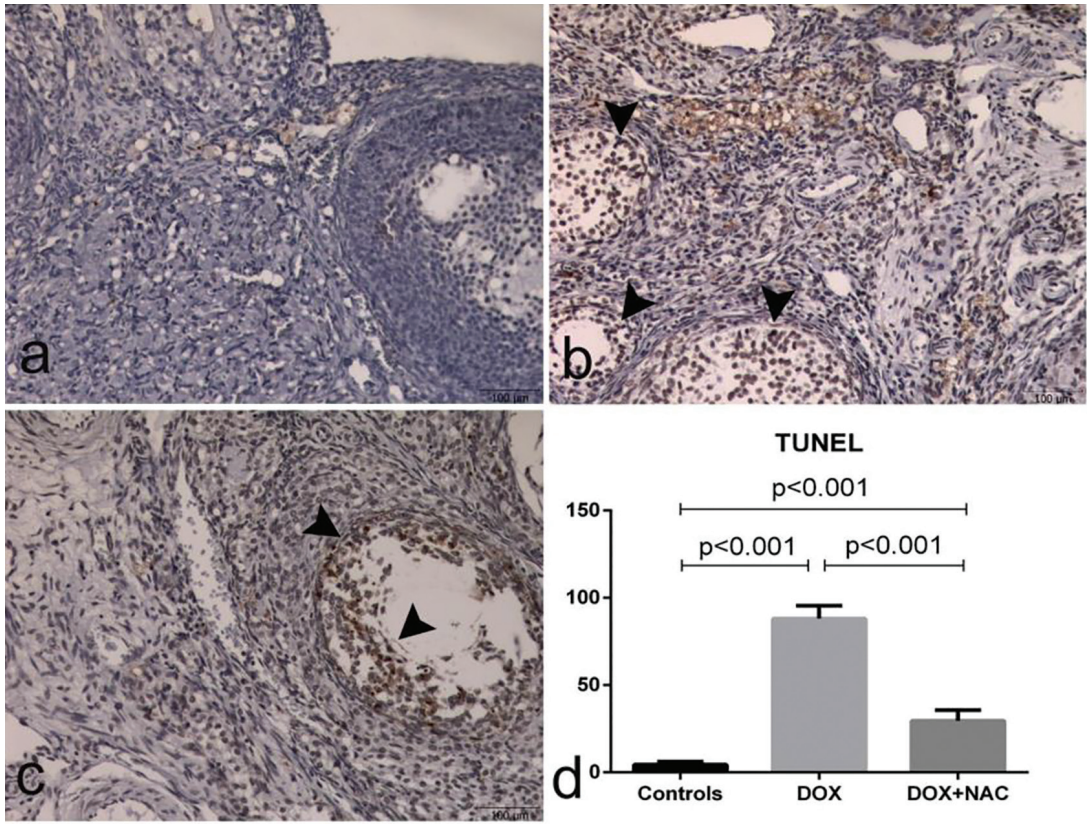

Figure 4. Effects of DOX and NAC treatments on granulosa cell apoptosis. **(a)** control, **(b)** DOX, **(c)** DOX+NAC, **(d)** distribution of TUNEL-positive cell percentages of groups, arrowhead; PCNA-positive cells, magnification; 200X

DOX: Doxorubicin, NAC: N-acetylcysteine, PCNA: Proliferating cell nuclear antigen

Table 2 summarizes the effect that DOX treatment significantly induces oxidative stress, inflammation, reduced antioxidant capacity, and apoptosis, as evidenced by changes in GSH, TNF-α, TAS, TOS, and Caspase-3 levels. NAC co-treatment effectively mitigates these adverse effects, restoring oxidative balance, reducing inflammation, and limiting apoptosis. These findings underscore the potential of NAC as a therapeutic agent to counteract DOX-induced ovarian toxicity.

Discussion

The present study demonstrated that DOX exerts profound gonadotoxic effects in female rats, whereas NAC provides significant protection against ovarian injury through its antioxidant, anti-inflammatory, and anti-apoptotic properties. Consistent with earlier reports that chemotherapy can deplete ovarian reserve and impair follicular integrity[1,19,20], our findings confirmed that DOX markedly reduced serum AMH levels (6.75±0.49 → 5.31±0.47 ng/mL; p<0.001) and the percentage of healthy follicles; while increasing the number of atretic follicles. Importantly, NAC co-treatment preserved ovarian reserve, restoring AMH to 6.51±0.78 ng/mL (p=0.004 vs. DOX), which is clinically relevant given that AMH is a robust marker of ovarian function and fertility potential.

In line with the mechanistic role of oxidative stress in DOX-induced toxicity[19,21], our biochemical results showed that DOX significantly depleted systemic and tissue antioxidants, lowering serum GSH (422.64±69.23 → 280.98±32.26 mg/L; p<0.001) and TAS (8.13±1.01 → 6.12±1.03 U/mL; p=0.001), while increasing TOS (7.18±1.00 → 11.84±2.55 U/mL; p=0.002). NAC administration reversed these alterations, with GSH increasing to 363.86±73.71 mg/L (p=0.018 vs DOX) and TAS to 7.56±0.59 U/mL (p=0.003), accompanied by normalization of TOS (7.88±1.12 U/mL; p=0.003). These findings corroborate previous work where natural antioxidants such as gallic acid[5], resveratrol[6], and quercetin[16] attenuated DOX- or cisplatin-induced ovarian damage via restoration of redox balance. The present study extends this evidence to NAC, an aminothiol with potent GSH-replenishing capacity[7,8].

Inflammation and apoptosis are pivotal mediators of chemotherapy-induced ovarian injury[3,19,22]. Here, DOX elevated serum TNF-α by 48% (175.87±25.91 → 260.77±18.08 ng/L; p<0.001) and tissue caspase-3 levels (2.06±0.29 → 3.14±0.33 ng/mL; p<0.001), consistent with enhanced inflammatory signaling and apoptotic cell death. NAC significantly mitigated these effects, lowering TNF-α to 184.55±30.00 ng/L (p<0.001 vs. DOX) and caspase-3 to 2.17±0.28 ng/mL (p<0.001 vs. DOX). Parallel histological findings revealed a reduction in

edema, hemorrhage, and infiltration scores, together with decreased TUNEL-positive granulosa cells and enhanced PCNA immunoreactivity in the NAC-treated group. These results are consistent with prior reports showing that NAC alleviated oxidative and apoptotic injury in γ-radiation-induced ovarian failure[11] and in ovarian torsion models[9], further confirming its cytoprotective potential in diverse ovarian injury settings.

Moreover, a very recent study, using ultrasonographic and histopathological evaluation, confirmed that longterm NAC administration (100 mg/kg for 21 days) attenuated DOX-induced ovarian and uterine toxicity, preserving follicular counts and AMH levels in rats[23]. Additionally, emerging evidence indicates that NAC can counteract chemotherapy-induced ferroptosis by enhancing GPX4, Nrf2, and HO-1 expression and suppressing lipid ROS accumulation[22]. These findings reinforce the translational promise of NAC as an ovoprotective agent.

Taken together, the histological, immunohistochemical, and biochemical evidence indicates that NAC confers multifaceted ovarian protection against DOX. By scavenging ROS, replenishing GSH, suppressing inflammatory mediators, and limiting apoptosis, NAC preserved follicular integrity and granulosa cell proliferation. Compared with studies on other antioxidants such as gallic acid[5], resveratrol[6], and rutin[10], our results highlight NAC as a potent candidate with translational potential, especially given its long-established clinical safety profile in other indications[7,8]. The translational relevance of these findings is noteworthy. Fertility preservation is a critical concern for young women undergoing chemotherapy[20,21], and strategies that can be co-administered safely alongside anticancer drugs are urgently needed. By maintaining AMH levels, follicular health, and proliferative capacity, NAC may offer a feasible adjunct to protect ovarian reserve during chemotherapy.

In conclusion, this study provides the first comprehensive evidence that NAC significantly alleviates DOX-induced ovarian toxicity by improving redox balance, reducing inflammation, and inhibiting apoptosis, thereby preserving ovarian reserve. These findings, supported by both quantitative outcomes and comparison with recent, relevant preclinical studies, suggest that NAC merits further evaluation as a fertility-preserving agent in oncological settings.

Study Limitations

Nevertheless, some limitations must be acknowledged. Estrous cycle staging was not performed, which may contribute to subtle variability in ovarian morphology and hormone levels. An NAC-only group was not included, preventing isolation of its independent effects. The study relied on a single DOX dose with short-term follow-up and a modest sample size (n=7/group). Furthermore, functional fertility outcomes, such as pregnancy rates and litter size, were not assessed. Future studies should evaluate dose–response and timing effects of NAC, incorporate long-term fertility outcomes, and explore additional mechanistic pathways including ferroptosis[22].

Conclusion

This study highlights the protective role of NAC in a rat model of DOX-induced ovarian failure. By reducing oxidative stress, inflammation, and apoptosis, NAC preserves ovarian structure and function, emphasizing its potential as a therapeutic agent to safeguard reproductive health in patients undergoing chemotherapy. Future studies should expand upon these findings by exploring different NAC doses and administration schedules, (pre, co-, and post-treatment with DOX) to determine optimal timing and efficacy. Longer-term experiments incorporating functional fertility outcomes such as estrous cyclicity, pregnancy rates, and litter size are warranted to establish the true reproductive benefits of NAC. Mechanistic investigations focusing on ferroptosis, mitochondrial dysfunction, and antioxidant signaling pathways (e.g., Nrf2, HO-1, GPX4) may clarify the molecular basis of NAC's protective effects. Additionally, strategies combining NAC with other antioxidants or established fertility-preserving agents should be assessed. Finally, translational studies in women undergoing chemotherapy are needed to evaluate the clinical applicability and safety of NAC for fertility preservation.

Ethics

Ethics Committee Approval: The current study followed the "Principles of laboratory animal care" (NIH publication no: 86-23, revised 1985) and, as well as the Aksaray University Experimental Animals Ethics Committee, and it was carried out in compliance with ethical standards (approval number: 14, date: 19.02.2024).

Informed Consent: Not necessary.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.Ö., İ.K., Concept: İ.Ö.A., H.E., Design: İ.Ö.A., M.Ö., Data Collection or Processing: M.Ö., İ.K., M.D., Analysis or Interpretation: İ.Ö.A., H.E., İ.K., M.D., Literature Search: İ.Ö.A., H.E., M.D., Writing: İ.Ö.A., H.E.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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