Clinical and Experimental Health Sciences

# N-Acetylcysteine Ameliorates 5-Fluorouracil-Induced Ovarian Injury in Rats

Elif Ayazoğlu Demir[1], Ahmet Menteşe[2], Hatice Küçük[3], Nihal Türkmen Alemdar[4,5], Selim Demir[6*], Yüksel Aliyazıcıoğlu[2]

[1] Karadeniz Technical University, Macka Vocational School, Department of Chemistry and Chemical Processing Technologies, Trabzon, Türkiye.
[2] Karadeniz Technical University, Faculty of Medicine, Department of Medical Biochemistry, Trabzon, Türkiye.
[3] University of Health Sciences, Trabzon Kanuni Training and Research Hospital, Department of Pathology, Trabzon, Türkiye.
[4] Karadeniz Technical University, Graduate School of Health Sciences, Department of Medical Biochemistry, Trabzon, Türkiye.
[5] Recep Tayyip Erdogan University, Vocational School of Health Services, Department of Medical Services and Techniques, Rize, Türkiye.
[6] Karadeniz Technical University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Trabzon, Türkiye.

**Correspondence Author:** Selim Demir

**E-mail:** selim-demir@hotmail.com

**Received:** 04.11.2022 **Accepted:** 05.07.2023

**ABSTRACT**

**Objective:** Although 5-fluorouracil (5-FU) is one of the most commonly used chemotherapeutics worldwide, it has been shown that 5-FU administration can cause reproductive toxicity in recent years. N-acetylcysteine (NAC) is the precursor of glutathione, the most important endogenous antioxidant molecule and is known for its effective antioxidant and anti-inflammatory properties. Although NAC is one of the most studied antioxidant molecules, its curative effect against ovarian damage caused by 5-FU has not been demonstrated to date. It was therefore aimed to investigate whether NAC is therapeutic against 5-FU-induced ovotoxicity in this study for the first time.

**Methods:** Rats were first exposed to a single dose of 5-FU (100 mg/kg) and then treated with NAC (10 and 20 mg/kg) for three days. The oxidative stress, inflammation and apoptosis markers in ovarian tissues were also determined using spectrophotometric methods. Ovarian tissues were also evaluated histologically.

**Results:** It was revealed that the levels of oxidative stress, inflammation and apoptosis biomarkers in ovarian tissue increased by 5-FU administration ($p<.005$). Treatments with NAC significantly restored these damages dose-dependently ($p<.005$). Moreover, these biochemical findings were confirmed by histological examination.

**Conclusion:** NAC can be considered as a potential therapeutic molecule against 5-FU-induced reproductive toxicity, as it can abolish the ovarian toxicity caused by 5-FU by reducing oxidative stress, inflammation and apoptosis.

**Keywords:** 5-Fluorouracil, Apoptosis, Inflammation, N-acetylcysteine, Oxidative stress, Ovotoxicity

## 1. INTRODUCTION

The incidence of cancer is increasing all over the world and chemotherapeutic drugs are frequently used in the treatment of cancer (1,2). Since chemotherapeutics have a low therapeutic index, they affect not only target malignant cells but also healthy cells (3). In young women, side effects of chemotherapeutics are seen on the reproductive system and gonadal damage, permanent ovarian failure and menopause may develop after chemotherapy (4). 5-fluorouracil (5-FU) is one of the most used chemotherapeutics (2). It is a drug that causes decreased DNA synthesis mainly by inhibiting thymidylate synthase. It also interferes with RNA processing and protein synthesis (5). It is frequently used against colorectal, breast, stomach, pancreatic, head and neck cancers (6). In addition to leukopenia, hemolytic anemia and thrombocytopenia, 5-FU administration may cause some side effects such as stomatitis, mucositis, diarrhea and various organ toxicity (2). Recent studies have shown that 5-FU, one of the most frequently used chemotherapeutics in the world, causes ovarian damage (5,7). Since there is no approved treatment protocol or a specific antidote to be used against the toxic effects of chemotherapeutic use, different toxicity prevention strategies are currently being studied meticulously (1,8).

N-acetylcysteine (NAC) is a water-soluble molecule and a precursor of glutathione (GSH) (9,10). It has been shown to has antioxidant, anti-inflammatory and anticancer activities (11,12). Although NAC has been shown to abolish the damage caused by various chemotherapeutics in liver, heart and kidney tissues (4,10,11), there is no research on its effect on 5-FU-induced ovotoxicity. Thus, we hypothesized that NAC may have therapeutic effects against 5-FU-induced




*DOI: 10.33808/clinexphealthsci.1199615*

ovarian damage, and in this study, we aimed to determine the effects of NAC against 5-FU-related ovarian damage in an experimental model for the first time.

## 2. METHODS

### *2.1. Animals*

A total of 30 healthy female Sprague-Dawley rats (weighing 200±20 g) were used to conduct the current study. All rats were kept in clean plastic cages at an ambient temperature of 22±2°C and subjected to a 12 h photo period of light-dark cycle. All experimental procedures were approved by Local Animal Research Ethics Committee of Karadeniz Technical University (Protocol No: 2021/30). The estrus stages of the rats were evaluated by vaginal smear and only the rats determined to be in estrus period were included in experiments (13).

### *2.2. Experimental Design*

The rats were divided into five groups. Control group was given physiological saline for four day. 5-FU group was given 5-FU (100 mg/kg) in 1st day and saline followed 3 days. 5-FU+NAC groups were given 5-FU in 1st day and NAC (10 mg/kg and 20 mg/kg, respectively) followed 3 days. *Per se* NAC group (only 20 mg/kg) was given physiological saline in 1st day and NAC (20 mg/kg) for 3 days. All the treatments were given via intraperitoneally (IP). All rats were sacrificed by cervical dislocation on day 5 (8,14) and their ovarian tissues were removed. Half of the tissues were stored at – 80 ℃ for biochemical examinations, while the other half were subjected to 10% formaldehyde fixation for histological analysis. Doses of 5-FU (15,16) and NAC (17,18) were determined according previous studies.

### *2.3. Biochemical Analysis*

Ovarian tissue samples (approximately 20 mg) were homogenized in PBS using a homogeniser. The protein levels were determined using a commercial colorimetric kit (Pierce BCA Protein Assay Kit, Thermo Scientific, Rockford, IL). Total oxidant status (TOS) and total antioxidant status (TAS) levels were determined using commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey). The ratio of TOS to TAS was accepted as oxidative stress index (OSI) and calculated the following formula (14):

$$\text{OSI (arbitrary unit)} = \frac{\text{TOS (μmoL hydrogen peroxide equivalent/L)}}{\text{TAS (mmoL trolox equivalent/L)}} \text{ x } 100$$

Malondialdehyde (MDA) levels were determined using previously described method (19). The tissue levels of oxidative stress [superoxide dismutase (SOD), glutathione (GSH), 8-hydroxy-2'-deoxyguanosine (8-OHdG)], inflammation [interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α)] and apoptosis (caspase-3) biomarkers were determined using ready-to-use ELISA kits (Fine Biotech Co. Ltd, Wuhan, China).

### *2.4. Histological Analysis*

Tissue samples were fixed in in 10% formaldehyde for two days and then placed in paraffin blocks after routine tissue follow-up. Sections of 5 μm were stained with H&E and examined under a light microscope. The prepared slides were evaluated using previously described scale (20,21). Analyzes were performed by a pathologist unfamiliar with the groups using the coding system.

### *2.5. Statistical Analysis*

A sample size of 6 animals per group has been provided the appropriate power (1–β=0.8) to identify significant differences in MDA (adjusted α=0.016 for two comparisons), taking into account an effect size d=2.0, a two-sided t-test, and a sample size ratio=1 (G*Power 3.1.9.2, Kiel University, Kiel, Germany). Statistical analyses were made by using the SPSS version 23.0 (Chicago, IL, USA) statistical package software. The Shapiro-Wilk test was used to check whether the data were normally distributed. Data were expressed as the median [interquartile range, 25-75% (IQR)] for non-normal distribution. As all of the MDA data fitted non-normal distribution, the Kruskal Wallis test was used for overall comparison of the groups. Comparisons between groups were performed using the Mann-Whitney U test with the Bonferroni correction. Regarding the Bonferroni correction, α=0.05/10 = 0.005 was established to have statistical significance.

## 3. RESULTS

All biochemical findings were presented in Table 1. Results indicated that 5-FU administration elevated MDA, TOS, OSI and 8-OHdG levels compared to control group (all p=.004). However, the levels of oxidative stress parameters in 5-FU+NAC (20 mg/kg) group were lower compared to 5-FU group (all p=.004).

The TAS, SOD and GSH levels of 5-FU group were lower than control group (all p=.004). In 5-FU+NAC (10 mg/kg) and 5-FU+NAC (20 mg/kg) groups, SOD and GSH levels were significantly higher than 5-FU group (all p=.004).

The IL-6, TNF-α and caspase-3 levels of 5-FU group were higher than control group (all p=.004). In 5-FU+NAC (10 mg/kg) group, only TNF-α levels was lower than 5-FU group (p=.004). The levels of all these parameters in 5-FU+NAC (20 mg/kg) group were lower compared to 5-FU group significantly (p=.004). Also, there was no difference between control group and *per se* (only 20 mg/kg NAC) group in terms of ovarian biochemical parameters (p>.005).

Histopathological features of ovarian tissues obtained from all groups were shown in Figure 1 and quantified in Table 2. Administration of 5-FU significantly increased vascular congestion and edema in the ovarian tissue compared with control group (p=.003 and p=.004, respectively). However, NAC (20 mg/kg) treatment alleviated the levels of vascular congestion significantly compared with 5-FU group (p=.003).

Also, there was no difference between control group and *per se* (only 20 mg/kg NAC) group in terms of ovarian histological findings (p>.005).

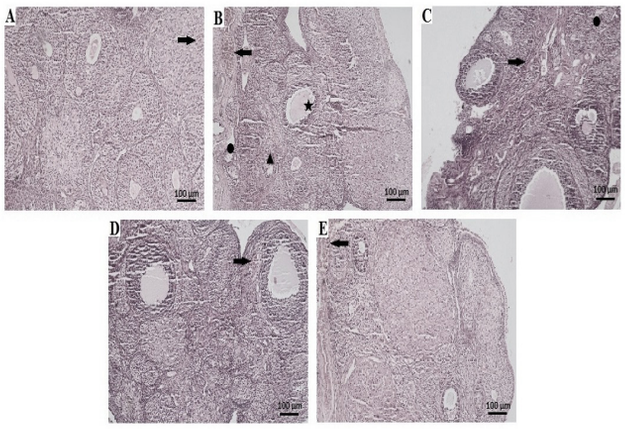


***Figure 1.*** *Histopathological examination of rat ovarian tissues stained with H&E (×100).* ***(A) Control group;*** *black arrow: vascular congestion.* ***(B) 5-FU group;*** *black arrow: vascular congestion, filled circle: edema, filled triangle: follicular degeneration, black star: leukocyte infiltration.* ***(C) 5-FU+10 mg/kg NAC group;*** *black arrow: vascular congestion, filled circle: edema.* ***(D) 5-FU+20 mg/kg NAC group;*** *black arrow: vascular congestion.* ***(E) 20 mg/kg NAC (per se) group;*** *black arrow: vascular congestion.*

## 4. DISCUSSION

Although chemotherapeutics are indispensable drugs in cancer treatment, their low selectivity is a problem waiting to be solved (14). It has been suggested that increased ROS, inflammation and apoptosis, and suppression of antioxidant system are main mechanisms in 5-FU-induced tissue damage (6,15). ROS formation induced by 5-FU increases lipid peroxidation (15). It is known that increased ROS causes protein and DNA damage, especially lipids in cells (22-24). MDA is considered the most important indicator of lipid peroxidation level (25). The higher MDA levels obtained in 5-FU group indicate that 5-FU induces lipid peroxidation in this study. NAC applications alleviated MDA formation dose-dependent manner. Consistently, it was previously reported that NAC could exert tissue protective activity by inhibiting holoxan, cisplatin, doxorubicin and cyclophosphamide-induced lipid peroxidation (4,10,26,27).

TOS and TAS measurement is a useful, fast and simple method to evaluate the complex oxidative mechanism of a pathology (28,29). Reduced GSH, a low molecular weight tripeptide, is an antioxidant molecule. It is known that increased GSH oxidation increases susceptibility to lipid peroxidation (27). The fact that 5-FU administration caused higher OSI levels

***Table 1.*** *Effects of NAC on biochemical parameters of ovarian tissues rats*

| | Control | 5-FU | 5-FU+NAC (10 mg/kg) | 5-FU+NAC (20 mg/kg) | NAC (20 mg/kg) |
|---|---|---|---|---|---|
| **TOS (µM $H_2O_2$ equivalent/L)** | 11.9 (10.8-12.6) | 34.8 (24.0-47.6)[a] | 18.1 (15.9-23.0) | 12.2 (11.5-12.7)[b] | 12.5 (12.2-13.3) |
| **TAS (mM trolox equivalent/L)** | 0.55 (0.51-0.57) | 0.30 (0.22-0.32)[a] | 0.33 (0.19-0.52) | 0.59 (0.35-0.66) | 0.51 (0.39-0.54) |
| **OSI (arbitrary unit)** | 2.24 (2.14-2.31) | 12.1 (10.3-14.2)[a] | 5.99 (4.28-8.61)[a] | 2.18 (1.87-3.36)[b] | 2.44 (2.35-3.34) |
| **MDA (nmol/mg protein)** | 16.9 (15.7-18.1) | 46.3 (44.8-48.5)[a] | 28.8 (19.9-48.7) | 16.2 (12.5-22.5)[b] | 18.4 (11.3-20.5) |
| **8-OHdG (ng/mg protein)** | 3.92 (3.63-4.88) | 8.47 (7.49-9.64)[a] | 6.77 (3.52-9.02) | 5.17 (4.27-5.87)[b] | 4.57 (3.04-5.36) |
| **SOD (ng/mg protein)** | 3.57 (2.44-3.76) | 0.99 (0.94-1.33)[a] | 2.17 (1.90-2.76)[b] | 3.10 (2.30-4.43)[b] | 2.87 (1.69-4.61) |
| **GSH (µg/mg protein)** | 12.5 (9.55-15.18) | 5.45 (5.1-6.33)[a] | 8.3 (7.55-9.28)[b] | 11.3 (9.95-14.55)[b] | 12.1 (9.23-16.4) |
| **IL-6 (pg/mg protein)** | 1166.3 (1029.7-1418.6) | 3192.7 (2750.3-3515.9)[a] | 2076.0 (1568.9-2646.4) | 1343.4 (708.2-1821.1)[b] | 1052.9 (748.2-1431.0) |
| **TNF-α (pg/mg protein)** | 142.9 (129.1-161.5) | 515.3 (476.4-569.4)[a] | 257.2 (163.8-355.8)[b] | 170.8 (143.8-182.5)[b] | 141.6 (100.1-164.3) |
| **Caspase 3 (ng/mg protein)** | 2.84 (2.43-3.41) | 7.45 (6.34-9.82)[a] | 5.37 (4.34-7.02) | 3.77 (2.84-4.90)[b] | 2.44 (1.68-3.29) |

*5-FU: 5-fluorouracil, NAC: N-acetylcysteine, TOS: total oxidant status, TAS: total antioxidant status, OSI: oxidative stress index, MDA: malondialdehyde, SOD: superoxide dismutase, GSH: glutathione, 8-OHdG: 8-hydroxy-2'-deoxyguanosine, IL-6: interleukin-6, TNF-α: tumour necrosis factor-alpha.*

*P-values according to Kruskal-Wallis variance analysis and followed the Mann-Whitney U test with the Bonferroni correction.*

*Data were expressed as medians with a 25th and 75th percentile interquartile range (IQR).*

*[a]p<.005 compared with control group, [b]p<.005 compared with 5-FU group.*

***Table 2.*** *Histopathological findings of experimental groups*

| | Control | 5-FU | 5-FU+NAC (10 mg/kg) | 5-FU+NAC (20 mg/kg) | NAC (20 mg/kg) |
|---|---|---|---|---|---|
| **Vascular congestion** | 1 (0-1) | 2.5 (2-3)[a] | 1 (1-2) | 1 (0-1)[b] | 1 (0-1.25) |
| **Edema** | 0 (0-0.25) | 2 (1-2)[a] | 1 (0.75-1) | 0 (0-1) | 0 (0-1) |
| **Hemorrhage** | 0 (0-0) | 1 (0-1) | 0 (0-0.25) | 0 (0-0) | 0 (0-0.25) |
| **Follicular degeneration** | 0 (0-0) | 1 (0-1) | 0 (0-1) | 0 (0-0.25) | 0 (0-0.25) |
| **Leukocyte infiltration** | 0 (0-0) | 1 (0-1) | 0 (0-1) | 0 (0-0.25) | 0 (0-0.25) |

*5-FU: 5-Fluorouracil, NAC: N-acetylcysteine.*

*P-values according to Kruskal-Wallis variance analysis and followed the Mann-Whitney U test with the Bonferroni correction.*

*Data were expressed as medians with a 25th and 75th percentile interquartile range (IQR).*

*[a]p<.005 compared with control group, [b]p<.005 compared with 5-FU group.*

and lower GSH levels showed that oxidative stress mediated ovotoxicity. NAC treatment attenuated ovarian injury by modulating oxidative stress. Consistently, it was previously reported that NAC could exert tissue protective activity by modulating methotrexate, cisplatin and doxorubicin-induced oxidative stress (11,30-34).

DNA is one of the most important targets of ROS and shows increased 8-OHdG DNA damage. 8-OHdG levels in ovarian tissues were therefore measured to determine the level of free radical-mediated DNA damage in this study (20). Elevated 8-OHdG levels in the 5-FU group indicated that DNA damage mediates ovotoxicity. Especially, NAC (20 mg/kg) application to 5-FU administered rats restored these changes. Similar with our results, it has been reported that NAC can exert a renoprotective effect against cisplatin-induced damage by inhibiting DNA damage (35).

SOD is one of the most important enzymes protecting the cell against ROS attacks (25), and 5-FU is known to increase tissue damage by suppressing SOD levels (6). Our results showed that systematic 5-FU administration suppressed SOD expression, while NAC treatments abolished this inhibition in dose-dependently. Consistently, it was previously reported that NAC could exert tissue protective effect against chemotherapeutic-induced tissue injury via modulating antioxidant enzymes (26,32,34).

Inflammation has been suggested as a second mechanism in 5-FU-induced toxicity (7). TNF-α and IL-6 are considered two of the main inflammatory cytokines (31). The elevated levels of these markers in 5-FU-treated rats indicates that 5-FU toxicity is mediated by inflammation. NAC applications to 5-FU administered rats restored these changes with its previously demonstrated anti-inflammatory activity (8,11,20). Consistently, it was previously reported that NAC could exert tissue protective effect against chemotherapeutic-induced injury via modulating inflammation (31,36).

Apoptosis is proceed by the activation of caspases, which are cysteine-dependent aspartate specific protease (21). Increased oxidative stress and inflammation levels can trigger apoptosis through caspase-3 activation (14). Therefore, it has been reported that compounds with anti-apoptotic properties may be useful agents in the prevention of chemotherapeutic-induced toxicity (21). The elevated caspase-3 levels in 5-FU-treated rats indicated that 5-FU toxicity is mediated by apoptosis. NAC (20 mg/kg) applications to 5-FU administered rats significantly restored these changes. These results were consistent with previous reports (35,37,38).

Histological evaluation results showed signs of increased vascular congestion and edema in the ovarian tissue of rats treated with 5-FU. Treatment with NAC (20 mg/kg) reversed 5-FU-induced ovarian injury. Similar with our findings, it is reported that NAC exhibits ovoprotective effect against ischemia/reperfusion (I/R) injury. In the same study, histological examination showed that NAC treatment improves edema, vascular congestion, hemorrhage and follicular degeneration findings caused by I/R damage (20).

Our study has a few limitations. First, the therepeutic effect of two concentrations of NAC were evaluated in this study. It was determined that 20 mg/kg dose of NAC could restore biochemical and histological changes better than 10 mg/kg dose of NAC against 5-FU induced ovarian damage. Therefore, future studies should evaluate the ovoprotective effect of NAC at doses above 20 mg/kg. Second, the molecular mechanisms involved in 5-FU-induced ovotoxicity or the ovoprotective effects of NAC in rats have not been investigated in detail. Therefore, further studies are needed to explain in detail how NAC improves 5-FU-related ovotoxicity. Third, the outcome of 5-FU and NAC administration in rats may not reflect the overall effects of such therapy in patients.

## 5. CONCLUSION

This study showed that NAC (especially the dose of 20 mg/kg) may have therapeutic effects against 5-FU-induced ovarian damage. This effect is thought to be mediated, at least in part, by antioxidant and anti-inflammatory activities of NAC. However, the use of NAC against 5-FU-induced ovotoxicity needs to be supported by more extensive *in vivo* and clinical studies.

***Acknowledgement:*** *This research was carried out using the existing facilities of Karadeniz Technical University (Trabzon, Türkiye).*
***Funding:*** *The author(s) received no financial support for the research.*
***Conflicts of interest:*** *The authors declare that they have no conflict of interest.*
***Ethics Committee Approval:*** *This study was approved by Local Animal Research Ethics Committee of Karadeniz Technical University (approval date 01.06.2021 and number 2021/30)*
***Peer-review:*** *Externally peer-reviewed.*
***Author Contributions:***
*Research idea: EAD, AM*
*Design of the study: EAD, AM, HK*
*Acquisition of data for the study: EAD, AM, HK, NTA, SD*
*Analysis of data for the study: EAD, AM, SD*
*Interpretation of data for the study: EAD, AM, SD*
*Drafting the manuscript: EAD, SD*
*Revising it critically for important intellectual content: AM, YA*
*Final approval of the version to be published: EAD, AM, HK, NTA, SD, YA*

## REFERENCES

[1] Zhang QY, Wang FX, Jia KK, Kong LD. Natural product interventions for chemotherapy and radiotherapy-induced side effects. Frontiers in Pharmacology 2018; 9: 1253. DOI: 10.3389/fphar.2018.01253.

[2] Al-Hamdany MZ, Al-Hubaity AY. Protective effects of N-acetylcysteine against 5-fluorouracil-induced pulmonary toxicity in albino rats. Iraqi Journal of Medical Sciences 2019; 12(2): 139-149. DOI: 10.31351/vol32iss1pp40-44.

[3] Lamberti M, Porto S, Marra M, Zappavigna S, Grimaldi A, Feola D, Pesce D, Naviglio S, Spina A, Sannolo N, Caraglia M. 5-Fluorouracil induces apoptosis in rat cardiocytes through intracellular oxidative stress. Journal of Experimental & Clinical Cancer Research 2012; 31(1): 60. DOI: 10.1186/1756-9966-31-60.

[4] Helal MAM. The effects of N-acetyl-L-cysteine on the female reproductive performance and nephrotoxicity in rats. Renal Failure 2016; 38(2): 311-320. DOI: 10.3109/0886022X.2015.112.7742.

[5] Lambouras M, Liew SH, Horvay K, Abud HE, Stringer JM, Hutt KJ. Examination of the ovotoxicity of 5-fluorouracil in mice. Journal of Assisted Reproduction and Genetics 2018; 35: 1053-1060. DOI: 10.1007/s10815.018.1169-6.

[6] Polk A, Vistisen K, Vaage-Nilsen M, Nielsen DL. A systematic review of the pathophysiology of 5-fluorouracil-induced cardiotoxicity. BMC Pharmacology and Toxicology 2014; 15: 47. DOI: 10.1186/2050-6511-15-47.

[7] Stringer JM, Swindells EOK, Zerafa N, Liew SH, Hutt KJ. Multidose 5-fluorouracil is highly toxic to growing ovarian follicles in mice. Toxicological Sciences 2018; 166(1): 97-107. DOI: 10.1093/toxsci/kfy189.

[8] Gunturk I, Yazici C, Kose K, Dagli F, Yucel B, Yay A. The effect of N-acetylcysteine on inflammation and oxidative stress in cisplatin-induced nephrotoxicity: A rat model. Turkish Journal of Medical Sciences 2019; 49: 1789-1799. DOI: 10.3906/sag-1903-225.

[9] Kazaz IO, Demir S, Yulug E, Colak F, Bodur A, Yaman SO, Karaguzel E, Mentese A. N-acetylcysteine protects testicular tissue against ischemia/reperfusion injury via inhibiting endoplasmic reticulum stress and apoptosis. Journal of Pediatric Urology 2019; 15(3): 253.e1-253.e8. DOI: 10.1016/j.jpurol.2019.02.005.

[10] Rosic G, Srejovic I, Zivkovic V, Selakovic D, Joksimovic J, Jakovljevic V. The effects of N-acetylcysteine on cisplatin-induced cardiotoxicity on isolated rat hearts after short-term global ischemia. Toxicological Reports 2015; 2: 996-1006. DOI: 10.1016/j.toxrep.2015.07.009.

[11] Akbulut S, Elbe H, Eris C, Dogan Z, Toprak G, Otan E, Erdemli E, Turkoz Y. Cytoprotective effects of amifostine, ascorbic acid and N-acetylcysteine against methotrexate-induced hepatotoxicity in rats. World Journal of Gastroenterology 2014; 20(29): 10158-10165. DOI: 10.3748/wjg.v20.i29.10158.

[12] Elsayed A, Elkomy A, Elkammar R, Youssef G, Abdelhiee EY, Abdo W, Fadl SE, Soliman A, Aboubakr M. Synergistic protective effects of lycopene and N-acetylcysteine against cisplatin-induced hepatorenal toxicity in rats. Scientific Reports 2021; 11(1): 13979. DOI: 10.1038/s41598.021.93196-7.

[13] Mentese A, Alemdar NT, Livaoglu A, Demir EA, Aliyazicioglu Y, Demir S. Suppression of cisplatin-induced ovarian injury in rats by chrysin: An experimental study. Journal of Obstetrics and Gynaecology 2022; 42(8): 3584-3590. DOI: 10.1080/01443.615.2022.2130201.

[14] Demir EA, Mentese A, Kucuk H, Alemdar NT, Demir S. *p*-Coumaric acid alleviates cisplatin-induced ovarian toxicity in rats. Journal of Obstetrics and Gynaecology Research 2022; 48(2): 411-419. DOI: 10.1111/jog.15119.

[15] Demir EA, Mentese A, Demir S, Kucuk H, Alemdar NT, Aliyazicioglu Y. Evaluation of therapeutic effect of chrysin against 5-fluorouracil-induced ovarian damage in rats. Farabi Medical Journal 2023; 2(1): 1-7.

[16] Zhang S, Liu Y, Xiang D, Yang J, Liu D, Ren X, Zhang C. Assessment of dose-response relationship of 5-fluorouracil to murine intestinal injury. Biomedicine & Pharmacotherapy 2018; 106: 910-916. DOI: 10.1016/j.biopha.2018.07.029.

[17] Samuhasaneeto S, Thong-Ngam D, Kulaputana O, Patumraj S, Klaikeaw N. Effects of N-acetylcysteine on oxidative stress in rats with non-alcoholic steatohepatitis. Journal of the Medical Association of Thailand 2007; 90(4): 788-797.

[18] Li WH, Wang L, He HY, Chen J, Yu YR. Expression of neutrophil gelatinase-associated lipocalin in low osmolar contrast-induced nephropathy in rats and the effect of N-acetylcysteine. Experimental and Therapeutic Medicine 2016; 12(5): 3175-3180. DOI: 10.3892/etm.2016.3779.

[19] Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analitical Biochemistry 1978; 86(1): 271-278. DOI: 10.1016/0003-2697(78)90342-1.

[20] Ersoy GS, Eken M, Tal R, Oztekin D, Devranoglu B, Kaygusuz EI, Cevik O. N-acetylcysteine leads to greater ovarian protection than enoxaparin sodium in a rat ovarian torsion model. Reproductive BioMedicine Online 2016; 33: 93-101. DOI: 10.1016/j.rbmo.2016.03.009.

[21] Demir EA, Mentese A, Livaoglu A, Alemdar NT, Demir S. Ameliorative effect of gallic acid on cisplatin-induced ovarian toxicity in rats. Drug and Chemical Toxicology 2023; 46(1): 97-103. DOI: 10.1080/01480.545.2021.2011312.

[22] Turan I, Demir S, Aliyazicioglu R, Kilinc K, Yaman SO, Cakiroglu KA, Kanbolat S, Demir EA, Mentese A, Aliyazicioglu Y, Deger O. Dimethyl sulfoxide extract of *Dianthus carmelitarum* induces S phase arrest and apoptosis in human colon cancer cells. Nutrition and Cancer 2019; 71(7): 1181-1188. DOI: 10.1080/01635.581.2019.1598563.

[23] Mahmoud AM, Hozayen WG, Ramadan SM. Berberine ameliorates methotrexate-induced liver injury by activating Nrf2/HO-1 pathway and PPAR-γ, and suppressing oxidative stress and apoptosis in rats. Biomedicine & Pharmacotherapy 2017; 94: 280-291. DOI: 10.1016/j.biopha.2017.07.101.

[24] Aliyazicioglu Y, Demir S, Yaman SO, Sener SO, Demir EA, Aliyazicioglu R, Turan I. Phytochemical analysis of *Dorycnium pentaphyllum* and its antiproliferative effect on cervix cancer cells. KSU Journal of Agriculture and Nature 2019; 22(Suppl 2): 365-373. DOI: 10.18016/ksutarimdoga.vi.579938.

[25] Demir S, Kazaz IO, Kerimoglu G, Demir EA, Colak F, Yilmaz S, Mentese A. Astaxanthin protects testicular tissue against torsion/detorsion-induced injury via suppressing endoplasmic reticulum stress in rats. Journal of Investigative Surgery 2022; 35(5): 1044-1049. DOI: 10.1080/08941.939.2021.1995540.

[26] Bulucu F, Ocal R, Karadurmus N, Sahin M, Kenar L, Aydin A, Oktenli C, Koc B, Inal V, Yamanel L, Yaman H. Effects of N-acetylcysteine, deferoxamine and selenium on doxorubicin-induced hepatotoxicity. Biological Trace Element Research 2009; 132: 184-196. DOI: 10.1007/s12011.009.8377-y.

[27] Mansour HH, El-Kiki SM, Hasan HF. Protective effect of N-acetylcysteine on cyclophosphamide-induced cardiotoxicity in rats. Environmental Toxicology and Pharmacology 2015; 40(2): 417-422. DOI: 10.1016/j.etap.2015.07.013.

[28] Kazaz IO, Demir S, Kerimoglu G, Colak F, Alemdard NT, Dogan SY, Bostan S, Mentese A. Chlorogenic acid ameliorates torsion/detorsion-induced testicular injury via decreasing endoplasmic reticulum stress. Journal of Pediatric Urology 2022; 18(3): 289.e1-289.e7. DOI: 10.1016/j.jpurol.2022.02.013.

[29] Demir EA, Demir S, Kazaz IO, Kucuk H, Alemdar NT, Buyuk A, Mentese A, Aliyazicioglu Y. Arbutin abrogates testicular ischemia/reperfusion injury in rats through repression of inflammation and ER stress. Tissue and Cell 2023; 86: 102056. DOI: 10.1016/j.tice.2023.102056.

[30] Abdel-Wahab WM, Moussa FI, Saad NA. Synergistic protective effect of N-acetylcysteine and taurine against cisplatin-induced nephrotoxicity in rats. Drug Design, Development and Therapy 2017; 11: 901-908. DOI: 10.2147/DDDT.S131316.

[31] Abdel-Wahab WM, Moussa FI. Neuroprotective effect of N-acetylcysteine against cisplatin-induced toxicity in rat brain by modulation of oxidative stress and inflammation. Drug Design, Development and Therapy 2019; 13: 1155-1162. DOI: 10.2147/DDDT.S191240.

[32] Cetinkaya A, Bulbuloglu E, Kurutas EB, Kantarceken B. N-acetylcysteine ameliorates methotrexate-induced oxidative liver damage in rats. Medical Science Monitor 2006; 12(8): 274-278.

[33] Kockar MC, Nazıroglu M, Celik O, Tola HT, Bayram D, Koyu A. N-acetylcysteine modulates doxorubicin-induced oxidative stress and antioxidant vitamin concentrations in liver of rats. Cell Biochemistry and Function 2010; 28: 673-677. DOI: 10.1002/cbf.1707.

[34] Zaki SM, Mohamed EA, Motawie AG, Fattah SA. N-acetylcysteine versus progesterone on the cisplatin-induced peripheral neurotoxicity. Folia Morphologica 2018; 77(2): 234-245. DOI: 10.5603/FM.a2017.0090.

[35] Luo J, Tsuji T, Yasuda H, Sun Y, Fujigaki Y, Hishida A. The molecular mechanisms of the attenuation of cisplatin-induced acute renal failure by N-acetylcysteine in rats. Nephrology Dialysis Transplantation 2008; 23: 2198-2205. DOI: 10.1093/ndt/gfn090.

[36] Kang KS, Shin S, Lee SI. N-acetylcysteine modulates cyclophosphamide-induced immunosuppression, liver injury, and oxidative stress in miniature pigs. Journal of Animal Science and Technology 2020; 62(3): 348-355. DOI: 10.5187/jast.2020.62.3.348.

[37] Goyal V, Bews H, Cheung D, Premecz S, Mandal S, Shaikh B, Best R, Bhindi R, Chaudhary R, Ravandi A, Thliveris J, Singal PK, Niraula S, Jassal DS. The cardioprotective role of N-acetyl cysteine amide in the prevention of doxorubicin and trastuzumab-mediated cardiac dysfunction. Canadian Journal of Cardiology 2016; 32(12): 1513-1519. DOI: 10.1016/j.cjca.2016.06.002.

[38] Kurauchi K, Nishikawa T, Miyahara E, Okamoto Y, Kawano Y. Role of metabolites of cyclophosphamide in cardiotoxicity. BMC Research Notes 2017; 10(1): 406. DOI: 10.1186/s13104.017.2726-2.

**How to cite this article:** Ayazoglu Demir E, Mentese A, Kucuk H, Turkmen Alemdar N, Demir S, Aliyazicioglu Y. N-Acetylcysteine Ameliorates 5-Fluorouracil-Induced Ovarian Injury in Rats. Clin Exp Health Sci 2023; 13: 776-781. DOI: 10.33808/clinexphealthsci.1199615