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The role of oxidative stress in chemotherapy-induced gonadotoxicity in a rat model, and the protective effects of *Nigella Sativa* oil on oxidative stress, the anti-Müllerian hormone level, and apoptosis

K. CETINKAYA[1], M. ATASEVER[2], Z. ERISGIN[3], C. SONMEZ[4], C. OZER[5], B. COSKUN[6], M. ALISIK[7]

[1]Department of Obstetrics and Gynecology, Health Sciences University, Ankara City Hospital, Ankara, Turkey
[2]Department of Obstetrics and Gynecology, Faculty of Medicine, Giresun University, Giresun, Turkey
[3]Department of Histology, Faculty of Medicine, Giresun University, Giresun, Turkey
[4]Department of Clinical Chemistry, Health Sciences University, Dr Abdurrahman Yurtarslan Oncology Training and Research Hospital, Ankara, Turkey
[5]Department of Physiology, Faculty of Medicine, Gazi University, Ankara, Turkey
[6]Department of Obstetrics and Gynecology, Health Sciences University Gulhane Training and Research Hospital, Ankara, Turkey
[7]Department of Biochemistry, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Turkey

Abstract. – OBJECTIVE: This study aimed to determine the role of oxidative stress (OS) in carboplatin-induced gonadotoxicity and whether Nigella Sativa oil (NSO), an herbal antioxidant, has a protective effect on ovarian apoptosis, OS, and the anti-Müllerian hormone (AMH) level in a rat model.

MATERIALS AND METHODS: The study included 24 adult female rats that were divided into 4 treatment groups. Group A saline + saline (sham group); group B: NSO + saline; group C: saline + carboplatin; group D: NSO + carboplatin. Saline, NSO, and carboplatin were administered intraperitoneally 24 and/or 48 h before sacrification as 4 mL/kg, 4 mL/kg, and 80 mg/kg, respectively. Apoptosis, OS parameters, and AMH were measured.

RESULTS: Oxidant levels and apoptosis were higher, whereas AMH and the antioxidants were lower in group C than in group A. Apoptosis, OS parameters, and AMH levels were negatively affected by chemotherapy (CTx) in group C whilst improvement in those parameters was observed in group D following NSO pretreatment. The levels of apoptosis and malondialdehyde (MDA), an OS parameter, in group D were lower than in group C as they declined from 34.3% to 8.65% ($p = 0.002$) and from 199.4 nmol/g tissue to 136.4 nmol/g tissue ($p = 0.002$), respectively. However, the slight increase in AMH level from 2.7 ng/mL to 3.5 ng/mL due to the NSO effect was not significant between groups C and D.

CONCLUSIONS: The present findings show that carboplatin has adverse effects on AMH, ovarian tissue apoptosis, and OS parameters. NSO pretreatment might protect ovarian tissue and decrease CTx-induced ovarian injury by decreasing OS and apoptosis, but the protective effect of NSO on AMH is limited.

Key Words:
Antioxidant, Apoptosis, Gonadotoxicity, *Nigella Sativa* oil, Oxidative stress, Ovarian reserve.

Introduction

Preserving fertility during and after treatment is very important for a special group of women with ovarian cancer (OC)[1]. Advances in cancer treatment modalities and considerable improvements in patient survival have increased hopes for fertility preservation of the reproductive age group with OC. Fertility-sparing surgery and adjuvant chemotherapy (CTx) are becoming more acceptable approaches to the treatment of OC in patients seeking pregnancy[2]; however, these treatments can be toxic to the ovaries, and premature ovarian failure (POF) is a significant risk following CTx[3]. This decrease in ovarian

Corresponding Author: Kadir Cetinkaya, MD; e-mail: kacetinkaya@gmail.com

reserve is a result of oocyte and granulosa cell damage[1,2]. Furthermore, CTx increases oxidative stress (OS), which leads to the development of POF[4]. The disruption of the balance between OS and antioxidant defense mechanisms might be responsible for ovarian toxicity and ovarian damage caused by CTx[5]. There is a need to identify treatment approaches that can decrease OS and/or increase antioxidant production in order to protect against the tissue damage-related side effects of CTx[1-3].

Many women use herbal products in addition to traditional CTx[2,3,6]. Herbal and traditional medicine are commonly used by patients with cancer during CTx, and are considered supplementary treatment methods in some countries[7,8]. However, the actual risks and benefits of these methods remain unknown, as several herb-drug interactions have not been supported by clinical studies[7].

The potential of several new agents to increase the effectiveness of platinum-based CTx and reduce its possible harmful side effects has been studied[9]. Therefore, the use of natural products in conjunction with platinum-based CTx appears to be a promising strategy[6]. It has been reported that CTx is strongly associated with such complications as infertility, ovarian dysfunction, and increased follicular apoptosis[4,5]; however, the mechanisms that cause these side effects are not fully understood. As such, experimental studies are needed to examine reproductive damage and decreased ovarian reserve in patients that undergo intensive CTx.

Nigella Sativa is a medicinal plant that has been the focus of recent research due to its antioxidant, anti-inflammatory, and anti-proliferative effects identified in preclinical models[7,10,11]. The protective effects of *Nigella Sativa* oil (NSO) have been studied, although it is still unknown how the gonadotoxic effects of carboplatin are affected by NSO. The present study aimed to determine the role of OS in carboplatin CTx-induced gonadotoxicity in a rat model and whether NSO has a protective effect on ovarian apoptosis, OS, and the anti-Müllerian hormone (AMH) level.

Materials and Methods

The study protocol was approved by the Experimental Animal Studies Ethics Committee, Ankara, Turkey.

Animals

Rats were obtained from Kobay Animal Laboratory, Ankara, Turkey. The study included 24 healthy adult female Wistar albino rats aged 3-4 months and weighing 250-300 g that were randomly divided into 4 treatment groups of 6 each. Rats in all but the sham group were administered CTx and/or NSO intraperitoneally (IP). Preoperatively, all rats were kept in polypropylene rat cages at 22ºC under a 12/12 h light/dark cycle and had access to rat chow and water *ad libitum*. All rats were euthanized on the day of surgery. The rats were treated in accordance with the Guide for the Care and Use of Laboratory Animals[12].

Drug Administration

Drug administration in all groups was performed 48 h and 24 h before surgery (Table I). Group A received IP saline (4 mL/kg) alone, whereas groups B, C, and D received an IP cytostatic agent and/or NSO. Ovarian failure was induced *via* the cytostatic regimen, which consisted of carboplatin (Carboplatin-Koçak, Koçak Farma, Istanbul, Turkey) administered to groups C and D 24 h before surgery at a dose of 80 mg/kg in 2 mL of saline. The rats in groups B and D also received an injection of NSO 4 mL/kg 48 h before surgery (Figure 1). Rats were divided into 4 treatment groups; group A: saline + saline (sham group); group B: NSO + saline; group C: saline + carboplatin; group D: NSO + carboplatin. NSO was prepared at 25ºC using the cold pressing

Table I. Experimental groups and applied treatments.

Groups	48 h before BO	24 h before BO	Surgery day
Group A	4 ml/kg of Saline	4 ml/kg of Saline	BO
Group B	4 ml/kg NSO	4 ml/kg of Saline	BO
Group C	4 ml/kg of Saline	80 mg/kg carboplatin	BO
Group D	4 ml/kg NSO	80 mg/kg carboplatin	BO

BO: Bilateral oophorectomy, NSO: Nigella Sativa oil.

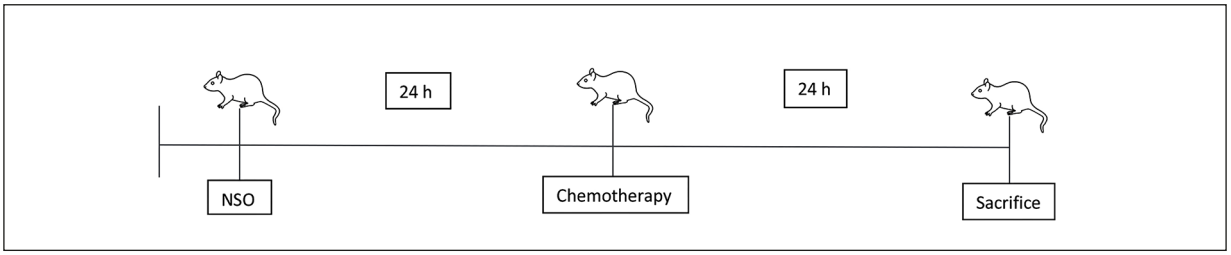

Figure 1. Time points of drug administration in group D.

method in order to determine its ability to reverse CTx-induced ovarian failure[10]. The NSO dose was determined based on previous studies[7,10].

Surgical Procedure

Surgery was performed under anesthetic and aseptic conditions. The rats were anesthetized *via* intramuscular (IM) injection of ketamine HCl 90 mg/kg (Ketalar, Pfizer, Istanbul, Turkey) and xylazin HCl 10 mg/kg (Rompun 2%, Bayer Türk, Istanbul, Turkey). A 3-cm midline incision was made to expose the abdominal cavity. The uterine horn and adnexa were localized. Bilateral oophorectomy was performed, and the ovaries were preserved for flow cytometry and biochemical analysis. Blood samples were taken from the left ventricle using an injector under anesthesia. Finally, the animals were euthanized at the end of the experiment to prevent suffering.

Blood Sampling and Tissue Preparation

Blood samples collected to evaluate ovarian damage were placed in tubes containing ethylenediamine tetraacetic acid (EDTA). The samples were then centrifuged for 10 min at 3,000 rpm, and the plasma was separated and stored at -80ºC. One of the ovaries was placed in neutral formalin, and contralateral ovarian tissue samples were quickly frozen in liquid nitrogen and preserved at -80ºC until analysis.

Biochemical Measurements

AMH, ischemia-modified albumin (IMA), albumin, thiol, and disulfide (SS) were measured in rat plasma. Glutathione (GSH) and malondialdehyde (MDA) levels were measured in ovarian tissue[13].

AMH Measurement

The serum AMH level was measured using an enzyme-linked immunosorbent assay (ELISA) kit (AnshLabs, TX, USA) and Biotek ELx800 microplate reader (Vermont, USA), according to the manufacturer's protocol. The measurement range was specified as 0.23-15 ng/ml.

Thiol and SS Measurement

Thiol and SS were measured by an automated spectrophotometric method using Roche cobas-c501 automated analyzer (Roche, Mannheim, Germany).

IMA Measurement

IMA level was measured with albumin cobalt binding test. 200 µL patient saline was mixed with 50 µL 0.1% cobalt chloride and incubated for 5 minutes. Meanwhile, it was ensured that cobalt was bound with albumin. After incubation, 50 µL dithiothreitol (DTT) (Sigma, 1.5 mg/ml H_2O) was added, and the mixture was mixed, which ensured that DTT created a colorful complex with cobalt not bound with albumin. This colorful complex was measured spectrophotometrically at 479 nm wavelength. The results were provided in absorbance units (ABSU).

Albumin Measurement

Albumin level was studied in Roche cobas-c501 automated analyzer (Roche, Mannheim, Germany) using Roche diagnostic (Roche Diagnostic, Mannheim, Germany) commercials kits.

MDA Level Assay in Tissue

MDA, as a lipid peroxidation (oxidative stress) marker, was examined by measuring the formation of thiobarbituric acid-reactive substances. Tissue samples were homogenized in cold TCA (1 gr tissue 10 ml 10% TCA). The homogenate was centrifuged at 3,000 rpm for 10 minutes. 0.67% (m/v) TCA equal to the amount of 750 ml of the supernatant was added and heated at 100 °C for 15 minutes. The absorbance of the samples was measured with ELISA reader at 535 nm wavelength.

GSH Level Assay in Tissue

Antioxidant GSH levels were studied with the modified Ellman method. The tissue samples were homogenized in TCA, and centrifuged at 3,000 rpm for 10 minutes, after the supernatant had been removed, the homogenate was added with 2 ml 0.3 M Na_2HPO_4 $2H_2O$. 0.2 ml dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added, and after mixing, the mixture was measured with ELISA reader at 412 nm wavelength.

Flow Cytometry

Flow cytometry was performed to detect apoptosis. After the ovaries were cleaned from the surrounding adipose tissue, they were suspended *via* mechanical disruption. The prepared cell suspension was filtered through a wire-mesh specimen to remove the remaining tissue fragments. A binding buffer was added to the filtered tissue solution, which was centrifuged and followed by incubation with Annexin V antibody (Apoptosis Detection Kit, BD Biosciences Pharmingen, USA). After the addition of the binding buffer, apoptotic Annexin V-positive cells were analyzed using a BD Accuri C6® flow cytometer (Apoptosis Detection Kit, BD Biosciences Pharmingen, San Diego, CA, USA)[14].

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows v. 20 (IBM Corp., Armonk, NY, USA). The Kruskal-Wallis test was performed for multiple group comparisons, and the Mann-Whitney U test was used to determine differences between the two groups. Post-hoc comparison was performed with Bonferroni correction. The level of statistical significance was set at $p < 0.05$.

Results

Biochemical parameters and apoptosis results are shown in Table II.

Biochemical Findings

The levels of the antioxidants albumin, thiols, and GSH were lower, and the levels of the oxidants MDA, SS, and IMA were higher in group than in group A. In group C, the oxidant levels increased due to the effect of CTx, whereas the antioxidant levels decreased.

Following the NSO pretreatment, OS was decreased in group D in contrast to group C, especially the MDA level was lower in the prior compared to the latter group; it lessened from 199.4 nmol/g tissue to 136.4 nmol/g tissue ($p = 0.002$). The AMH level was lower in group C than in group A. OS parameters and the AMH level were negatively affected by CTx. Post CTx, the AMH level was 2.7 ng/mL in group C, *vs.* 3.5 ng/mL in group D, but the difference was not significant.

Flow Cytometry Findings

As an indicator of apoptosis, annexin V positivity based on flow cytometry showed that the apoptotic rate was low in groups A and B, and high in CTx-applied groups C and D (Figure 2 and Table II). The apoptotic rate was significantly higher in group C than in group A ($p = 0.002$) and was the highest among all the groups due

Table II. Comparison of biochemical parameters and apoptosis.

Location	Property	Marker	Unit	Group A	Group B	Group C	Group D	*p*-value
Tissue	Oxidant	MDA	(nmol/g tissue) means ± SE	131.38 ± 4	135.65 ± 7.67	199.48 ± 14.98	136.48 ± 4.59	0.004
	Antioxidant	GSH	(µmol/g tissue) means ± SE	15.15 ± 1.59	17.25 ± 1.82	12.00 ± 0.57	15.40 ± 1.18	0.114
	Apoptosis	Annexin V	(%)	0.96 ± 0.15	0.41 ± 0.19	35.28 ± 3.84	8.48 ± 1.72	< 0.001
Plasma	Oxidant	IMA	(U/mL)	0.9 ± 0.09	0.97 ± 0.09	1.16 ± 0.24	1.22 ± 0.31	0.001
		SS	(µmol/L)	39.48 ± 37.29	28.69 ± 26.06	50.6 ± 48.21	37.04 ± 35.03	0.798
	Antioxidant	Albumin	(g/L)	3.68 ± 0.25	3.55 ± 0.33	2.46 ± 0.32	2.41 ± 1.02	< 0.001
		Thiol	(µmol/L)	188.95 ± 39.22	182 ± 41.85	60.5 ± 29.47	57.4 ± 43.98	0.001
	Ov. Reserve	AMH	(ng/mL)	11.28 ± 3.7	11.45 ± 3.09	2.7 ± 0.74	3.53 ± 1.83	0.001

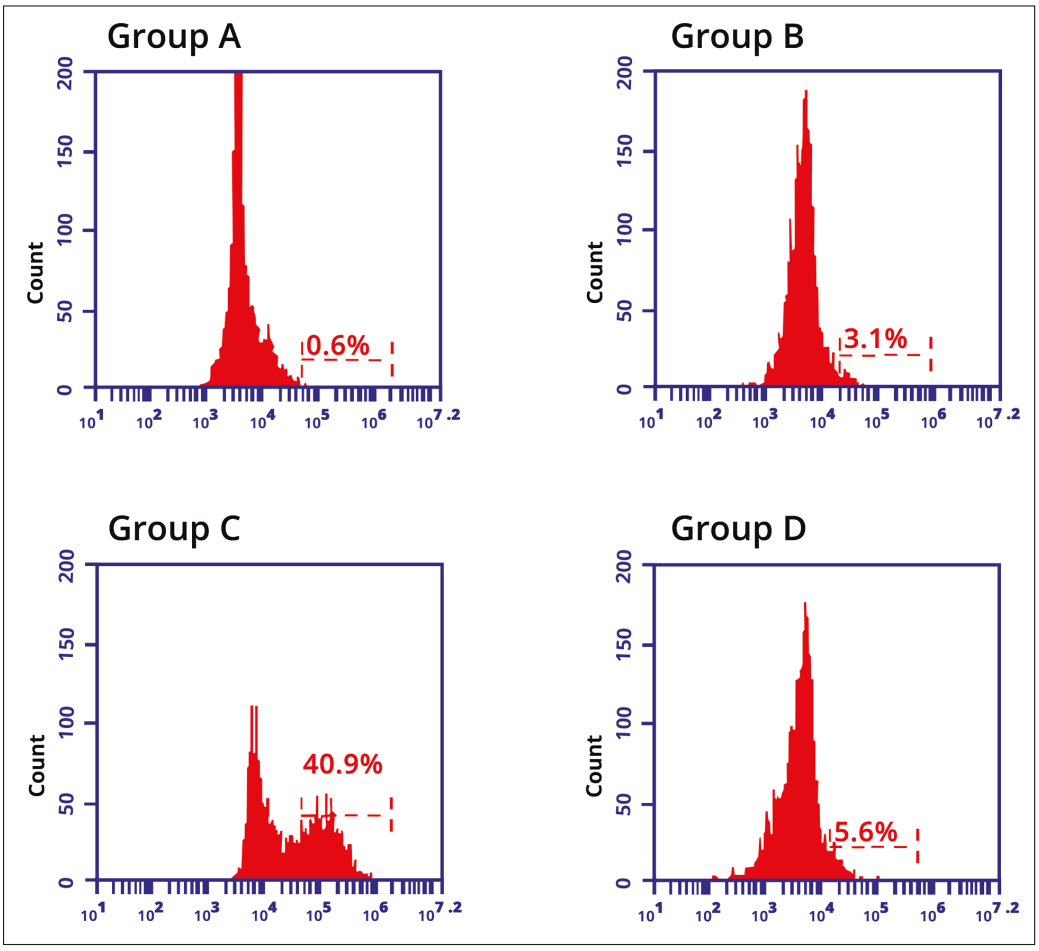

Figure 2. Histograms showing Annexin V positivity [the apoptotic rate (%)] in ovarian tissue.

to carboplatin administration. Following NSO pretreatment, the apoptotic rate was significantly lower in group D than in group C ($p = 0.002$).

Discussion

The present study examined the role of apoptosis and OS in CTx-induced ovarian injury in rats that were administered carboplatin CTx and whether such injury could be prevented by pretreatment administration of NSO. Carboplatin was administered IP, and its possible side effects were evaluated 24 h later. Deterioration of the balance of OS parameters in ovarian tissue and apoptotic damage, as well as a decrease in the AMH level, were observed following the administration of carboplatin CTx. Following injection of the herbal antioxidant NSO 24 h before carboplatin CTx, evident cytoprotective effects, a significant decrease in apoptosis, and improvement in OS parameters were observed.

As fertility in women of childbearing age can be adversely affected by anticancer drugs, fertility preservation is challenging and has attracted considerable attention among researchers aiming to provide a better quality of life for cancer survivors[1]. Furthermore, attempts are being made to preserve fertility prior to the commencement of cancer treatment, and cancer centers have developed fertility preservation programs for women of reproductive age[15]. Prior to the commencement of treatment, ovarian reserve is usually evaluated *via* AMH measurement[1]. AMH is produced by granulosa cells of primordial follicles in the ovarian cortex, which begins during the intrauterine period and gradually decreases during the course of women's reproductive years[15]. Therefore, the serum AMH level is the most accurate indicator for predicting the antral follicle pool, and a side

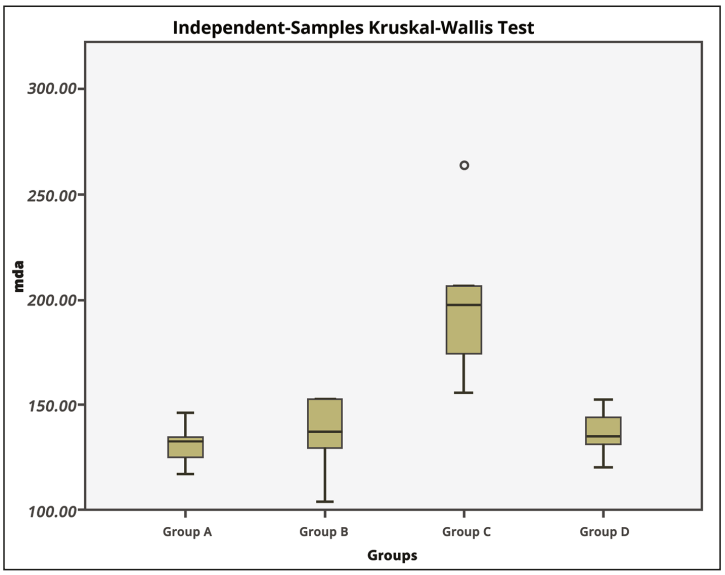

Figure 3. MDA levels in ovarian tissue.

effect of CTx is a rapid decrease in the AMH level[16].

Platinum-based anticancer drugs are used for OC treatment, as well as the treatment of other types of cancer. Morgan et al[9] and Meng et al[17] studied the cisplatin-induced ovarian damage mechanisms of ovarian follicle loss. The structures most affected by CTx damage in ovarian follicles were reported to be oocytes and granulosa cells[2,3]. Although the side effects of CTx can affect all systems, the most prominent side effects in young women of reproductive age are infertility, a decrease in ovarian reserve, and an increased prevalence of POF[1,2]. As the patient age or the CTx dose increases, the risk for these side effects also increases[16].

Following carboplatin CTx in the present study, ovarian reserve, OS parameters, and the apoptotic rate in ovarian tissue were negatively affected. Specifically, the AMH level and the levels of the antioxidants albumin, thiol, and GSH decreased following carboplatin CTx, whereas the levels of the oxidants MDA and IMA, and the apoptotic rate increased. Similarly, Wu et al[18] reported excessive follicle loss due to promotion of autophagy and apoptosis in granulosa cells in response to cisplatin toxicity. CTx can decrease the reproductive potential of ovarian tissue and neutrophil-secreted reactive oxygen species (ROS) significantly contribute to ovarian injury[4,5]. ROS damages cells *via* induction of lipid peroxidation[5,19]. The resulting intermediates, such as MDA, also cause oxidative injury to nucleic acids and cell proteins, exerting a destructive effect on cell membranes and altering cell integrity[19-21].

OS is a disturbance of the balance between ROS formation and the antioxidant defense system, which damages cellular molecules and, ultimately, tissues[4]. Carozzi et al[22] reported that OS plays a role in platinum-induced toxicity. Lipid peroxidation and DNA and protein damage can develop as a result of OS[19,21]. Low levels of ROS are essential for certain biochemical reactions and play a secondary role as a messenger in cell signaling[4,20]. Following CTx, ROS production is increased in the ovaries, which stimulates both OS and apoptosis[4,5]. It was reported that when OS is increased, the balance between ROS formation and destruction is disrupted, leading to DNA damage[1,17]. In particular, OS damage affects oocytes and granulosa cells[23]. Increased OS can increase primordial follicle activation and reduce ovarian reserve[17,20].

In addition to OS, apoptosis is also important, and excessive activation and apoptosis in the primordial follicle play a prominent role in CTx-induced ovarian damage[1,2]. Del Castillo et al[24] observed cell damage, especially DNA damage and apoptosis in follicles during exposure to chemotherapeutics in an adult mouse model of ovarian reserve and fertility. Similarly, a significant increase in apoptosis due to CTx was observed in the present study.

A decrease in the AMH level was observed following CTx in the present study, indicating that ovarian reserve and reproductive potential were negatively affected and that there is a need for approaches that prevent CTx damage and protect fertility. Moreover, it was observed that

the decrease in ovarian reserve due to CTx was accompanied by an increase in OS.

According to the literature, the use of antioxidants might help prevent the decrease in ovarian reserve and infertility caused by CTx-induced ovarian damage and increased OS[1,2,17]. Treatment with anti-inflammatory agents and antioxidant free radical scavengers can be used to prevent ovarian tissue damage[8,11]; therefore, inhibition of follicle activation or apoptosis pathways is important for minimizing ovarian damage[5,25,26]. Moreover, antioxidants inhibit both follicle apoptosis and primordial follicular activation[2,3], and resveratrol, curcumin, quercetin, and some natural antioxidants have been suggested for reducing ovarian damage[1,2,6].

NSO is a natural phytochemical with antioxidant and anti-proliferative properties[7,8,11]. In the present study, the increase in the level of MDA (a marker of increased OS) due to CTx was reduced following NSO pretreatment, but it didn't have a similarly positive effect on the AMH level. Although the post-CTx AMH level was 2.7 ng/mL in group C and 3.5 ng/mL in group D (NSO pretreatment), the slight increase between the two groups was not significant. Consistent with the present study, Meng et al[17] reported that a decrease in the MDA level following the administration of hydrogen-rich saline had a protective effect against cisplatin-induced ovarian damage. In addition, Ergin et al[25] reported that the use of sildenafil had a significant protective effect on follicle number and ovarian size in cases of cisplatin damage, but the same effect was not observed for AMH.

As such, the imbalance between antioxidant defense mechanisms and free radical production due to anticancer therapies has been associated with the pathogeneses underlying increased OS and ovarian damage[1]. The use of antioxidants can help prevent the adverse effects caused by CTx-induced ovarian damage[2]. The results of the present study show that NSO may have a protective effect against this ovarian damage, which manifested as significant improvement in OS parameters and apoptosis, but limited improvement in the AMH level. Improvement in the AMH level might depend on the dose and duration of both NSO and CTx. Additional research is required to clearly determine the optimal dosage and timing of both NSO and CTx.

The present experimental study administered all drugs to healthy rats; no tumor-bearing rats were used, which represents a study limitation.

Conclusions

OS parameters and apoptosis in ovarian tissue were assessed following CTx. The results suggested that ovarian injury following carboplatin administration is activated by ROS production and the impairment of the antioxidant defense system. Pretreatment with NSO resulted in a decreased level of MDA and apoptosis. NSO markedly decreased ROS production and apoptosis, thus reducing CTx-induced ovarian damage. In conclusion, pretreatment with NSO may prevent this damage by decreasing ROS production and supporting the balance in OS formation.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

Cetinkaya K.: A, C, D, F; Atasever M.: B, C, E, F; Erisgin Z.: B, C, E, F; Sonmez C.: B, C, F; Ozer C: B, C, F; Coskun B.: C, F; Alisik M.: B, C, F. A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article.

ORCID ID

Kadir Cetinkaya: 0000-0003-4490-8267
Melahat Atasever: 0000-0001-8232-4719
Zuleyha Erisgin: 0000-0003-3523-6542
Cigdem Sonmez: 0000-0001-9307-5674
Cigdem Ozer: 0000-0002-2705-4522
Bora Coskun: 0000-0002-2338-7186
Murat Alisik: 0000-0003-0434-3206

Ethics Approval

The study was approved by Kobay A.Ş. Animal Ethics Committee, Ankara, Turkey, Date: 21.10.2016, Approval Number: 200.

Informed Consent

Not applicable.

References

1) Spears N, Lopes F, Stefansdottir A, Rossi V, De Felici M, Anderson RA, Klinger FG. Ovarian damage from chemotherapy and current approaches to its protection. Hum Reprod Update 2019; 25: 673-693.
2) Roness H, Kalich-Philosoph L, Meirow D. Prevention of chemotherapy-induced ovarian damage: possible roles for hormonal and non-hormonal attenuating agents. Hum Reprod Update 2014; 20: 759-774.
3) Xiong J, Xue L, Li Y, Tang W, Chen D, Zhang J, Dai J, Zhou S, Lu Z, Wu M, Wang S. Therapy of endocrine disease: Novel protection and treatment strategies for chemotherapy-associated ovarian damage. Eur J Endocrinol 2021; 184: R177-R192.
4) Agarwal A, Gupta S, Sharma R. Oxidative stress and its implications in female infertility - a clinician's perspective. Reprod Biomed Online 2005; 11: 641-650.
5) Devine PJ, Perreault SD, Luderer U. Roles of reactive oxygen species and antioxidants in ovarian toxicity. Biol Reprod 2012; 86: 1-10.
6) Yang L, Chen Y, Liu Y, Xing Y, Miao C, Zhao Y, Chang X, Zhang Q. The Role of Oxidative Stress and Natural Antioxidants in Ovarian Aging. Front Pharmacol 2021; 11: 617843.
7) Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, Damanhouri ZA, Anwar F. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed 2013; 3: 337-352.
8) Butt AS, Nisar N, Mughal TA, Ghani N, Altaf I. Anti-oxidative and anti-proliferative activities of extracted phytochemical compound thymoquinone. J Pak Med Assoc 2019; 69: 1479-1485.
9) Morgan S, Lopes F, Gourley C, Anderson RA, Spears N. Cisplatin and doxorubicin induce distinct mechanisms of ovarian follicle loss; imatinib provides selective protection only against cisplatin. PLoS One 2013; 8: e70117.
10) Erisgin Z, Atasever M, Cetinkaya K, Akarca Dizakar SÖ, Omeroglu S, Sahin H. Protective effects of Nigella sativa oil against carboplatin-induced liver damage in rats. Biomed Pharmacother 2019; 110: 742-747.
11) Bordoni L, Fedeli D, Nasuti C, Maggi F, Papa F, Wabitsch M, De Caterina R, Gabbianelli R. Antioxidant and Anti-Inflammatory Properties of Nigella sativa Oil in Human Pre-Adipocytes. Antioxidants (Basel) 2019; 8: 51.
12) National Research Council. Guide for the Care and Use of Laboratory Animals. Washington, D.C.: National Academies Press; 2011 [cited 2022 Dec 28]. Available at: https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf.
13) Smith AM, Zeve DR, Grisel JJ, Chen WJ. Neonatal alcohol exposure increases malondialdehyde (MDA) and glutathione (GSH) levels in the developing cerebellum. Brain Res Dev Brain Res 2005; 160: 231-238.
14) Shapiro HM. Practical Flow Cytometry. Wiley; 2003.
15) Broer SL, Broekmans FJ, Laven JS, Fauser BC. Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update 2014; 20: 688-701.
16) Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. Endocr Rev 2009; 30: 465-493.
17) Meng X, Chen H, Wang G, Yu Y, Xie K. Hydrogen-rich saline attenuates chemotherapy-induced ovarian injury via regulation of oxidative stress. Exp Ther Med 2015; 10: 2277-2282.
18) Wu Y, Ma C, Zhao H, Zhou Y, Chen Z, Wang L. Alleviation of endoplasmic reticulum stress protects against cisplatin-induced ovarian damage. Reprod Biol Endocrinol 2018; 16: 85-97.
19) Casini AF, Ferrali M, Pompella A, Maellaro E, Comporti M. Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene-intoxicated mice. Am J Pathol 1986; 123: 520-531.
20) Lu J, Wang Z, Cao J, Chen Y, Dong Y. A novel and compact review on the role of oxidative stress in female reproduction. Reprod Biol Endocrinol 2018; 16: 80.
21) Aykaç G, Uysal M, Yalçin AS, Koçak-Toker N, Sivas A, Oz H. The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. Toxicology 1985; 36: 71-76.
22) Carozzi VA, Marmiroli P, Cavaletti G. The role of oxidative stress and anti-oxidant treatment in platinum-induced peripheral neurotoxicity. Curr Cancer Drug Targets 2010; 10: 670-682.
23) Takahashi A, Yousif A, Hong L, Chefetz I. Premature ovarian insufficiency: pathogenesis and therapeutic potential of mesenchymal stem cell. J Mol Med (Berl) 2021; 99: 637-650.
24) Del Castillo LM, Buigues A, Rossi V, Soriano MJ, Martinez J, De Felici M, Lamsira HK, Di Rella F, Klinger FG, Pellicer A, Herraiz S. The cyto-protective effects of LH on ovarian reserve and female fertility during exposure to gonadotoxic alkylating agents in an adult mouse model. Hum Reprod 2021; 36: 2514-2528.
25) Ergin B, Uluutku GB, Kale İ, Topal CS, Toğrul C, Muhcu M. Protective effects of sildenafil administration on chemotherapeutic-induced ovarian damage in rats. Can J Physiol Pharmacol 2022; 100: 486-491.
26) Luderer U. Ovarian toxicity from reactive oxygen species. Vitam Horm 2014; 94: 99-127.