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Effects of Diosmin Administration on Cisplatin-Induced Premature Ovarian Failure in A Rat Model

Sisplatin ile İnduklenen Prematür Over Yetmezliği Sıçan Modelinde Diosmin Uygulamasının Etkileri

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Abstract

Aim: We aimed to examine the potential beneficial effects of diosmin administration on cisplatin-induced premature ovarian failure (POF) in a rat model

Material and Method: Twenty-eight rats were divided into four groups. Group A rats (n:7) were determined as the sham group. The remaining rats received an intraperitoneal injection of 1.5 mg/kg/day cisplatin for 10 days to create a POF model. Then, they were randomly divided into 3 subgroups. Group B was determined as POF group. Group C rats were given 100mg/kg/day diosmin for 10 days simultaneously while creating POF model. Group D rats were given 100 mg/kg/day diosmin for 10 days after POF model was created. Twentieth day blood samples were taken and left ovaries were resected for examination.

Results: CIS-induced rats showed reduced levels of Superoxide Dismutase (SOD), Anti-Mullerian Hormone (AMH) and Estradiol (E2) compared to sham group rats ($p<0.05$). SOD, AMH and E2 values were not significantly different ($p>0.05$) among the sham group, group C and D. No significant ($p>0.05$) difference in Follicle Stimulating Hormone (FSH) value was observed among group C, D and sham groups. There was no significant ($p>0.05$) difference in the number of secondary and antral follicles among group C and D compared to the sham group. Primordial follicle count was significantly higher in group C than group B ($p<0.05$). Primary and total follicle count in group D was significantly ($p<0.05$) higher than group B.

Conclusion: Diosmin administration after chemotherapy or in combination with chemotherapy has shown helpful efficacy in maintaining the ovarian reserve and the structure of the follicles.

Keywords: Anti-mullerian hormone, diosmin, ovarian follicle, primary ovarian insufficiency

Öz

Amaç: Sisplatin kaynaklı erken yumurtalık yetmezliği (POF) modelinde diosmin uygulamasının biyokimyasal ve histopatolojik potansiyel yararlı etkilerini incelemeyi amaçladık.

Gereç ve Yöntem: Yirmi sekiz sıçan dört gruba ayrıldı. A Grubu sıçanlar (n:7) plasebo grubu olarak belirlendi. Kalan yirmi bir sıçana, POF modeli oluşturmak için 10 gün boyunca 1,5 mg/kg/gün sisplatin intraperitoneal olarak uygulandı. Daha sonra rastgele 3 alt gruba ayrıldılar. Grup B, sisplatine bağlı yumurtalık yetmezliği grubu olarak belirlendi. POF modeli oluşturulurken C grubu sıçanlara aynı anda 10 gün süreyle 100 mg/kg/gün diosmin verildi. D grubuna ise POF modeli oluşturulduktan sonra 10 gün süreyle 100 mg/kg/gün diosmin verildi. Yirminci günün sonunda Anti-Müllerian Hormon (AMH), Folikül Uyarıcı Hormon (FSH), Süperoksit Dismutaz (SOD) ve Estradiol (E2) değerlerinin incelenmesi için kan örnekleri alındı. Ayrıca histopatolojik inceleme için sol overler rezeke edildi.

Bulgular: Sisplatin ile indüklenen grupta, diğer gruplara kıyasla daha düşük SOD, AMH ve E2 seviyeleri izlendi ($p<0,05$). SOD, AMH ve E2 değerleri sham grubu, grup C ve grup D arasında anlamlı farklılık göstermedi ($p>0,05$). Grup C, grup D ve sham grupları arasında FSH değerlerinde anlamlı ($p>0,05$) fark gözlenmedi. Grup C ve D arasında sham grubuna göre sekonder folikül ve antral folikül sayısında anlamlı ($p>0,05$) fark saptanmadı. Primordial folikül sayısı grup C'de grup B'ye göre anlamlı derecede yüksekti ($p<0,05$). Primer folikül ve toplam folikül sayısı grup D'de grup B'ye göre anlamlı ($p<0,05$) daha yüksekti.

Sonuç: Diosmin tedavisi serum östrojen, AMH ve SOD düzeylerini artırırken FSH düzeylerini düşürdü. Ayrıca foliküller üzerinde önemli bir koruyucu etki göstermiştir. Kemoterapi sonrası veya kemoterapi ile kombinasyon halinde diosmin uygulanması, yumurtalık rezervinin ve foliküllerin yapısının korunmasında yardımcı etkinlik sağlamaktadır.

Anahtar Kelimeler: Antimüllerian hormon, diosmin, overyan folikül, primer yumurtalık yetmezliği

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INTRODUCTION

Premature ovarian failure (POF) is a primary ovarian defect characterized by premature depletion of ovarian follicles before the age of 40 years.[1] Chemotherapy-induced ovarian failure (COF) is defined as a state of impairment of both endocrine and reproductive ovarian function after exposure to chemotherapy.[2] COF is one of the etiological factors of POF. Although there is no clear definition of COF, irreversible amenorrhea lasting more than 12 months after chemotherapy and a follicle stimulating hormone (FSH) level ≥30 MIU/mL are usually diagnostic.[3] Studies show that one out of every 10,000 women before the age of 20, one in every 1,000 women before the age of 30, and one out of every 100 women before the age of 40 are diagnosed with POF.[4]

With the increase in cancer detection rates in recent years, the incidence of cancer at a young age is increasing. Accordingly, new approach modalities have started to gain importance in order to preserve fertility in women exposed to chemotherapy. In the United States, it is estimated that about 2% of women under the age of 40 have cancer. Unfortunately, due to the chemotherapy administered to these women, it leads to inevitable problems such as decreased ovarian reserves and COF.[5] COF development decreases the quality of life by causing hot flashes, osteoporosis, night sweats, vaginal dryness, sexual dysfunction and menopausal symptoms besides infertility.[6] For this reason, it is becoming more and more important to protect women against iatrogenic infertility caused by chemotherapy.

The incidence of COF varies with patient age, pre-treatment hormone levels, cytotoxic agent, duration of treatment and cumulative dose. For example, the mean rate of chemotherapy-induced amenorrhea after chemotherapy administration is 68% (95% confidence interval.[CI], 66% to 70%).[7]

Diosmin is a flavone glycoside derived from hesperidin found in citrus fruits. There are many in vitro and in vivo studies showing the antioxidant, antihyperglycemic, anti-inflammatory, antimutagenic, antibacterial, antihyperlipidemic, antifibrotic, anti-cancer, anti-proliferative, anti-metastatic and antiulcer properties of diosmin. This glycoside with its appropriate safety profile offers a reliable and an effective treatment option for many diseases.[8,9]

Although there are various alternatives to protect ovarian function and fertility in women diagnosed with cancer at a young age and undergoing chemotherapy, there is no definitive preventive method and research on this continues. We planned to investigate the fertility protective roles of diosmin in COF. We performed histopathological and biochemical evaluation to evaluate ovarian function and reserve. Thus, a new treatment modality can be created in the case of COF, which can be seen even in adolescence and causes infertility.

MATERIAL AND METHOD

The study was carried out with the permission of Maltepe University Animal Use and Care Ethics Committee (Date: ..... , Decision No: 2021.01.02) and performed in accordance with the Helsinki Declaration of World Medical Association recommendations on animal studies. The rats were Wistar Albino type, female, 12 weeks and weighing between 188-216 grams. Rats were placed in individual cages and fed ad libitum in an environment at a 20-22° temperature, 50%-55% humidity and 12-hour light/12-hour dark cycles.[10] The rats were given 10 days to get used to the environment before the study. Seven rats were reserved for the sham group, and the COF model was created with the remaining 21 rats.

Rats were given an intraperitoneal injection of 1.5 mg/kg/day cisplatin (Cisplatin-Kocak 50 mg, Turkey,) for 10 days to establish a COF model.[11] After 10 days, they were randomly divided into 3 subgroups (n: 6 per subgroup) to determine the effects of diosmin administration on COF concurrent and after chemotherapy.

Group A: Sham Group

5ml/kg/day saline was administered intraperitoneally to this group for 10 days. They were monitored for the next 10 days.

Group B: Cisplatin Induced Ovarian Insufficiency Group

The COF model was created and then they were monitored for 10 days.

Group C: Cisplatin Induced Ovarian Insufficiency+Concurrent Diosmin Administration Group

While creating the COF model, 100mg/kg/day of diosmin was administered to this group simultaneously for 10 days by oral gavage.[12,13] They were monitored for the next 10 days.

Group D: Cisplatin Induced Early Ovarian Failure+Diosmin Administration Group

After establishing the COF model, 100 mg/kg/day of diosmin was administered to this group by oral gavage for 10 days.[12,13]

At the end of the twentieth day, following anesthesia intracardiac blood samples were taken from all rats and their left ovaries were resected. Euthanasia was applied after the procedure. The rats were anesthetized with intraperitoneal injection of xylazine hydrochloride (Rompun, Bayer, Germany) and ketamine hydrochloride (Ketalar, Eczacıbası, Turkey).[14]

Biochemical Evaluation

Blood samples were centrifuged at 3000 g for 10 minutes immediately after collection in yellow capped tubes (BD Diagnostics) to obtain serum. Serum samples were stored at -80°C until measurement. Anti-mullerian hormone (AMH) (Bioassay Technology Laboratory, China), Estradiol (Elabscience, USA), FSH (Bioassay Technology Laboratory, China) and Superoxide Dismutase (SOD) (Bioassay Technology Laboratory, China) were measured by Enzyme-linked Immunosorbent Assay (ELISA). Readings were made by a microplate reader (Biotek Synergy Reader).

Histopathologic Evaluation

Ovarian samples were fixed in 10% formalin for 48 hours, dehydrated in ethanol series, cleaned and embedded in paraffin. The paraffin blocks were sectioned at a thickness of 5 mm using a sliding microtome (Leica RM2125RTS Nussloch Germany). Sections were stained with haematoxylin and eosin and analyzed using light microscope (Nikon Eclipse E600 microscope) by an experienced pathologist. Primordial, primary, secondary and antral follicles were counted in the largest part of the ovary to evaluate the ovarian reserve.[15]

Statistical Analysis

In the descriptive statistics of the data, mean, standard deviation, median, lowest, highest, frequency and ratio values were used. The distribution of variables was measured with the Kolmogorov-Smirnov test. ANOVA (Tukey test), Kruskal-Wallis and Mann-Whitney U tests were used in the analysis of quantitative independent data. SPSS 28.0 program was used in the analysis.

RESULTS

The mean weight of the rats included in the study was 201.7±7.1 (188-216) grams. There was no significant difference among the weights of the rats in the groups (p> 0.05) (**Table 1**).

Table 1. Comparison of biochemical and histopathologic features among groups

		Group A	Group B	Group C	Group D	p
SOD	Mean±sd	2.9±0.7	2.1±0.4	2.5±0.5	2.4±0.5	0.040[A]
	Median	2.8	2.1	2.7	2.7	
FSH	Mean±sd	5.3±0.6	8.7±1.8	7.6±1.7	7.0±1.7	0.004[A]
	Median	5.6	8.8	7.4	7.0	
AMH	Mean±sd	3.4±0.4	2.5±0.5	3.0±0.5	3.0±0.3	0.013[A]
	Median	3.4	2.6	2.9	3.0	
Estradiol	Mean±sd	6.7±1.8	3.3±0.9	4.9±1.5	5.2±1.8	0.004[A]
	Median	5.7	3.4	4.7	4.7	
Primordial follicle count	Mean±sd	12.0±3.9	4.3±1.4	7.3±1.9	7.1±2.7	0.001[K]
	Median	10.0	5.0	8.0	7.0	
Primary follicle count	Mean±sd	10.0±2.1	3.0±1.6	4.6±2.2	5.7±3.1	0.002[K]
	Median	11.0	3.0	4.0	5.0	
Secondary follicle count	Mean±sd	4.4±0.5	2.9±0.9	3.6±1.3	4.0±1.2	0.035[K]
	Median	4.0	3.0	3.0	4.0	
Antral follicle count	Mean±sd	8.0±2.6	4.3±1.5	6.1±1.6	6.0±1.3	0.008[A]
	Median	8.0	4.0	6.0	6.0	
Total follicle count	Mean±sd	34.4±7.7	14.4±3.4	21.6±5.4	22.9±5.3	0.000[A]
	Median	31.0	14.0	21.0	22.0	
Weight	Mean±sd	202.3±8.8	200.3±5.9	201.4±7.5	202.9±7.2	0.922[A]
	Median	203.0	199.0	201.0	206.0	

A ANOVA/K Kruskal-Wallis (Mann-Whitney U Test)

CIS-induced rats showed reduced levels of SOD, AMH and E2 compared to sham group rats (p< 0.05). SOD, AMH and E2 values were not significantly different (p> 0.05) among the sham group, group C and group D. FSH value was significantly lower in the sham group than CIS-induced rats (p< 0.05). No significant (p> 0.05) difference in FSH value was observed among group C, group D and sham groups (**Table 1**).

The number of all follicle groups was significantly (p< 0.05) higher in the sham group than in the COF group. There was no significant (p> 0.05) difference in the number of secondary and antral follicles among group C and group D compared to the sham group. Primordial follicle count was significantly higher in group C than group B (p< 0.05). Primary and total follicle count in group D was significantly (p< 0.05) higher than group B (**Table 1**, **Figure 1**).

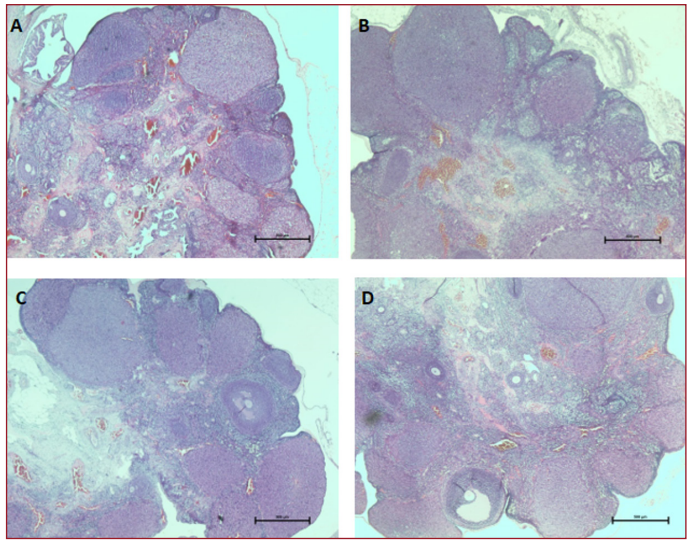

Figure 1. Histopathologic Evaluation

DISCUSSION

In our study, we investigated the protective effect of diosmin use on ovarian reserve in cases of POF that occurred after chemotherapy, as the primary aim. We demonstrated the positive effects of diosmin application on ovarian reserve both biochemically and histologically.

Studies have shown that cisplatin induces ovarian failure through follicular apoptosis. In the formation of cisplatin-induced premature ovarian failure; an increase in follicle atresia, a decrease in the number of follicles, and the mechanism of inducing apoptosis change in the granulose cells of the middle follicles play a role.[16] Although many treatment methods have been tried to prevent cisplatin-induced ovarian toxicity and preserve fertility, a definite therapeutic or preventive modality has not been established.

In 1925, diosmin found in the wort plant and since that time is used in the treatment of many diseases. It has been used for many years as a natural treatment method for varicose veins, hemorrhoids, venous insufficiency, leg ulcers and other circulatory problems.[17] Many in vitro and even in vivo studies to date have revealed the antioxidant, antihyperglycemic, anti-inflammatory, antimutagenic, antibacterial, antihyperlipidemic, antifibrotic, anti-cancer, anti-proliferative, anti-metastatic and antiulcer properties of diosmin. Studies

showing the effects of diosmin on cancer pathophysiology have shown that it can trigger premature aging in various cancer cell types. Studies to date have shown that diosmin has dose-dependent pro-apoptotic effects in colon, prostate, breast, urinary bladder and oral tumors.[8]

Free radicals formed due to oxidative stress are removed from biological systems by various antioxidants. SOD is involved in the defense against injury mediated by reactive oxygen species. These proteins, which are a group of metalloenzymes, take part in the catalysis of the dismutation of superoxide and they also reduce the level of superoxide, which can damage cells when they are in excessive concentration.[18] Studies in aflatoxin-induced liver and kidney damage model, doxorubicin-induced nephrotoxicity model, streptozotocin – nicotinamide-induced diabetes model and alloxan-induced diabetic nephropathy models have shown that SOD levels increase with diosmin treatment. In our study, similar to these studies, we showed that SOD levels increased with the use of diosmin concurrent or after chemotherapy.[19-22]

The menstrual cycle and ovulation in women depend on the production of gonadotropins (FSH and LH) by the pituitary gland. FSH stimulates the growth of granulosa cells of follicles growing in the ovary, thereby stimulating the production of estradiol by the follicles. High FSH levels are one of the most important indicators of female fertility and are widely used as an ovarian reserve test. The increase of FSH level in women receiving chemotherapy is indicative of decreased ovarian reserve and follicular depletion in women.[23] Besides, in contrast to increased FSH value, decreased estradiol level is also associated with POF. Estrogen deficiency is responsible for many of the clinical findings of POF such as hot flashes, vaginal dryness, night sweats, sleep disturbance and dyspareunia. In our study, we found that diosmin decreased FSH values and increased estradiol values in both groups, in which it was administered simultaneously and after chemotherapy. Considering the effect of diosmin on FSH and E2, we can interpret it as providing a protective effect on ovarian reserve and making menopausal clinical symptoms less felt. Thus, the quality of life of the person will increase due to fewer clinical symptoms.

AMH is a member of the transforming growth factor beta family that causes regression of Mullerian ducts. In today's clinical practice, AMH level is accepted as the best serum marker of ovarian reserve. It is expressed by the granulosa cells of follicles growing in the ovary from the primary stage to the minor antral stage.[24] When the AMH values were compared, the AMH value of the sham group were found to be significantly higher than COF group ($p< 0.05$). There was no significant difference in terms of AMH between the two diosmin-administered groups (group C and D) and the sham group ($p> 0.05$). We found that diosmin administration simultaneously or after cisplatin administration caused a positive increase in AMH value. Based on this increase, we demonstrated the protective effect of diosmin on ovarian reserve.

The number of follicles in the ovaries is the most important parameter of the length of ovarian life, ovarian reserve and fertility. The ovaries contain all the oocytes necessary for ovulation throughout the reproductive period.[25] When we examined the number of primary follicles and total follicles, we found that diosmin administration after chemotherapy was more effective. When we examined the secondary follicle and antral follicle numbers, we found that both diosmin administered groups (group C and D) showed similar results to the sham group. Some researchers have stated that follicle numbers may not give accurate results as an indicator of ovarian reserve in animal experiments. They argued that it is not a definitive indicator of the viability of damaged follicles that were not included in the count because they were damaged.[26] We attributed the differences in the number of follicles to this reason.

As the incidence of cancer increases over time, it is expected that women will face more POF due to chemotherapy treatments in the future. We hope that with diosmin and similar supportive treatments, women's fertility can be preserved and the negative symptoms of early menopause will be felt less.

Limitations Of The Study

The main limitation of our study was small size of our series and the lack of serial blood measurement in this experimental model. In addition, the absence of a group in which diosmin was administered simultaneously and after chemotherapy was determined as another limitation of our study.

CONCLUSION

Diosmin administration after chemotherapy or concurrently with chemotherapy ensures the preservation of the ovarian reserve and the structure of the follicles, and also this support causes oxidation-inhibiting effectiveness. This study provided valuable evidence for the prevention of cisplatin-induced ovarian toxicity of diosmin administration in rats.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Maltepe University Animal Ethics Committee (Date: ..... , Decision No: 2021.01.02).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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